FINAL REPORT

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Document 1 – Work Program Final Report

Date of Report: July 1, 2002

I. PROJECT TITLE: ***Evaluate establishment, impact of leafy spurge biocontrol agents.***

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Total Biennial Project Budget:

LCMR:	$ 140,000.00
LCMR Amount Spent:	$ 140,000.00
LCMR Balance:	$ 0.00

A. Legal Citation: ML 1999, Chap. 231 Sec.16 Subd. 16(b) Exotic Species
Language:
Evaluate Establishment, Impact of Leafy Spurge Biocontrol Agents $70,000 the first year and $70,000 the second year are from the trust fund to the commissioner of Agriculture to study flea beetles introduced to control leafy spurge by site characterization and assessment for biological control. This appropriation is available until June 30, 2002, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.
B. Status of Match Requirement: (None)

II. and III. FINAL PROJECT SUMMARY.

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge.

The results suggest that *Aphthona lacertosa* is the most effective species in controlling leafy spurge in Minnesota. *Aphthona lacertosa* has established at 100% of the release sites and significantly reduced leafy spurge by 63% across all sites studied. *Aphthona nigriscutis* established at 73% of the study sites, but at significantly lower densities. *Aphthona nigriscutis* most likely contributed to the control success at sites where both species occurred. Other introduced *Aphthona* species are difficult to locate in Minnesota and contributed little to the overall control success. Correlations between biotic/abiotic factors and flea beetle density were not clearly evident. *Aphthona nigriscutis* was observed at highest densities in dry sites with either sand or sandy loam soils.

Interspecific competition between *A. lacertosa* and *A. nigriscutis* was not affecting flea beetle populations. Small release quantities (<500 beetles) may have contributed to lack of establishment on early releases made in Minnesota. Although the beetles became established, all treatment populations were small one year after release. This suggests that the current practice of releasing >4,000 flea beetles per site will increase establishment, reproduction and eventual redistribution.

Phenology models were developed for predicting peak emergence. The model results can be used in two ways. First, the lower developmental threshold (LDT) and accumulated degree-days (ADD) to peak emergence can be used to calculate current ADD with local weather station temperature data. This allows resource managers to track degree-day accumulations and plan collection events on or near the predicted ADD for each species. The second method is to use maps developed on 30-year temperature data with estimated peak abundance dates in the field.

IV. OUTLINE OF PROJECT RESULTS:

Summary of Results 1, 2 and 3. (Please refer to Document 3 – Detailed Research Addendum for additional information).

BUDGET UPDATE:

Result 1:	**LCMR Budget**	**$ 56,523**
	Balance 6/30/01	$ 19,044
	Balance12/31/01	$ 10,040
	Balance 7/01/02	$ -0-
Result 2:	**LCMR Budget**	**$ 58,089**
	Balance 6/30/01	$ 31,186
	Balance 12/31/01	$ 14,885
	Balance 7/01/02	$ -0-
Result 3:	**LCMR Budget**	**$ 25,388**
	Balance 6/30/01	$ 20,188
	Balance 12/31/01	$ 16,593
	Balance 7/01/02	$ -0-

Objectives of this research include:

1) Assess the relationships between establishment of *Aphthona* spp. and biotic/abiotic factors.
2) Test for interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis.*
3) Test the effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis*.
4) Develop phenological models for *Aphthona* spp using accumulated degree-days.

Objective 1. Assess relationship between establishment of *Aphthona* flea beetles and biotic and abiotic factors.

Results:

To effectively utilize the variety of available biological control agents against leafy spurge, it is important to understand which biotic and abiotic factors influence the success of establishment and integration within spurge communities. Although there is some information in this area, a comprehensive look at current insect releases, establishment rates and environmental factors (both density dependent and density independent) will provide valuable information towards control efforts. Armed with this knowledge, biocontrol agents can be applied into situations where they have the greatest chance to succeed.

Table 1. Number of *Aphthona* spp. released for control of leafy spurge in Minnesota.
(Data summarized from MN Department of Agriculture leafy spurge database)

YEAR	No. of Releases	No. Insects Released
1989	8	4,500
1990	5	3,000
1991	5	4,000
1992	4	1,700
1993	3	2,000
1994	2	1,550
1995	13	14,250
1996	67	91,000
1997	158	1,511,153
1998	362	1,584,350
1999	439	4,650,322
2000	436	7,162,400
2001	539	6,797,870

Twenty-six sites were selected in three counties with 16 sites in Clay, 8 sites in Otter Tail, and 2 sites in Becker counties.

Table 2. Site characteristic information collected from each release site sampled.

Characteristics	Categories			
Site Type	open field/prairie	shrub-prairie mix	wet prairie	woodland/meadow
Shade	none	slight (5-30%)	moderate (31-60%)	heavy (>60%)
Water Drainage	well drained	moderately drained	poorly drained	
Topography	flat	valley or swale	hillside	hilltop
Slope	level	slight slope	steep slope	
Slope Direction	north	south	east	west
Soil Texture	multiple categories			

The most successful *Aphthona* species in moist, loamy sites has been the combination of *A. czwalinae* and *A. lacertosa*. It is known that *A. lacertosa* is the dominant species where it is released. Their populations tend to build up quickly, potentially affecting establishment of the other introduced *Aphthona* species, where mixed releases occur. In many of the release sites, it is unknown how well the other *Aphthona* species would establish in the absence of *A. lacertosa*.
Despite a range of microclimates, we were not able to associate *Aphthona* spp. abundance with biotic or abiotic factors with one exception. It was reported that soil texture influences *Aphthona* spp. abundance (Rees et al. 1996, Lym 1998 and Nowierski et al. 2002). Our study shows that *Aphthona lacertosa* abundance may be negatively affected by soils with high sand content. Nowierski et al. (2002) suggested that *A. lacertosa* in Europe are associated with sites containing higher levels of silt and clay. We found no relationship between *A. nigriscutis* and soil texture. This is in contrast to previous research that shows an apparent relationship of *A. nigriscutis* to sandy soils and xeric conditions (Rees et al. 1996, Lym 1998, and Nowierski et al. 2002).

The lack of association with biotic and abiotic factors may suggest that *A. lacertosa* may be able to establish on a wide variety of site types. This seems to be the case in our study where *A. lacertosa* established in 100% of the sites with a wide range of population densities. This is supported by previous research that suggested *A. lacertosa* can establish in hydric, mesic, and moderately dry sites (Fornasari 1996, Gassmann 1996, Rees et al. 1996, and Nowierski et al. 2002). For *A. nigriscutis*, we cannot make this same assumption. *Aphthona nigriscutis* established in 73% of the sites but the numbers were very small and are only a fraction of *A. lacertosa* densities. The extreme low densities of all but one of the *A. nigriscutis* populations most likely affected the regression analysis. It is our impression from field observations however, that *A. nigriscutis* prefers the drier sandy sites. Rees et al. (1996) and Nowierski et al. (2002) both suggest this pattern of *A. nigriscutis* preferring drier sites with sandier soils.

The sex ratio for *A. lacertosa* and *A. nigriscutis* was approximately 50/50. This is of particular interest for *A. nigriscutis* because sex ratios for this species in western states were found to strongly favor females. It is thought that a bacteria, *Wolbachia* spp., is lethal to male *A. nigriscutis* and thus may be a barrier to establishment (D. Kazmer pers.comm.). It would be of interest to know if *A. nigriscutis* populations in Minnesota are infected with *Wolbachia* spp.

Aphthona lacertosa and/or *A. nigriscutis* have shown that they can reduce leafy spurge densities. *Aphthona lacertosa* density appears to follow a classical biological control model where the population is small shortly after release, increases exponentially, peaks then decreases as the food source (spurge) decreases. *Aphthona lacertosa* populations tended to increase then peak shortly after spurge density began to decrease (Fig. 1). For many sites, the maximum *A. lacertosa* density was reached in 2000 then dropped in 2001. There were insufficient numbers of *A. nigriscutis* to discern general trends.

Figure 1. Mean spurge density for sites by number of years after *A. lacertosa* release compared to the relative *A. lacertosa* density by number of years after release. The relative *A. lacertosa* density is a ratio of the mean number of *A. lacertosa* per sweep by year.

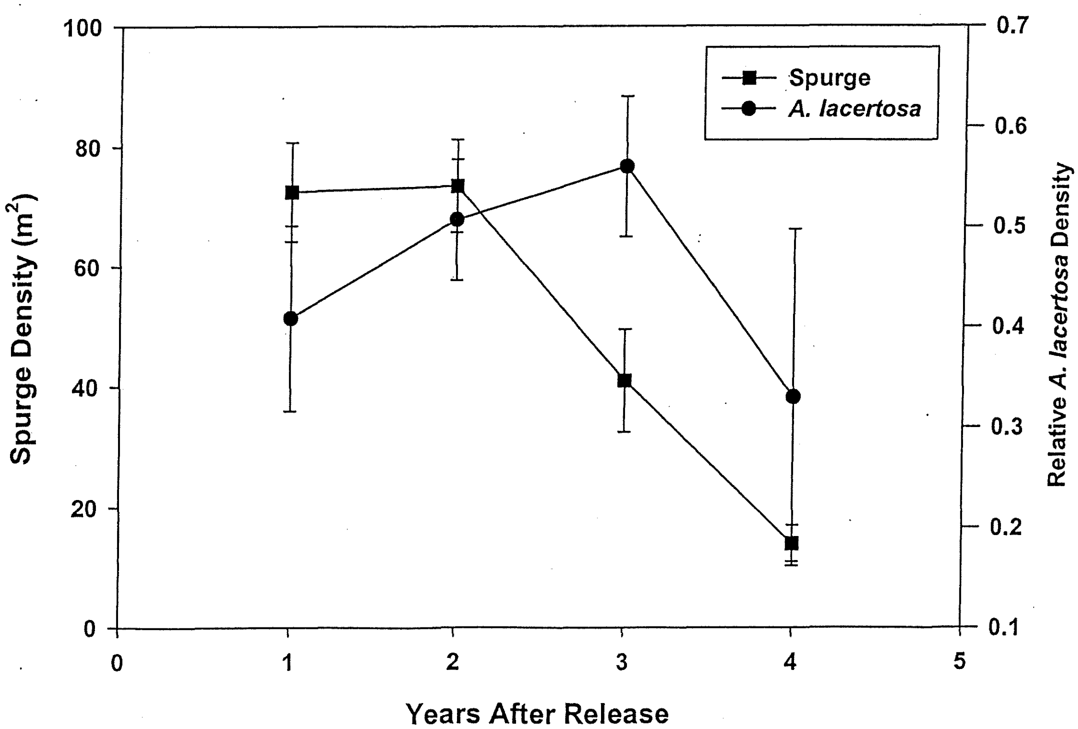
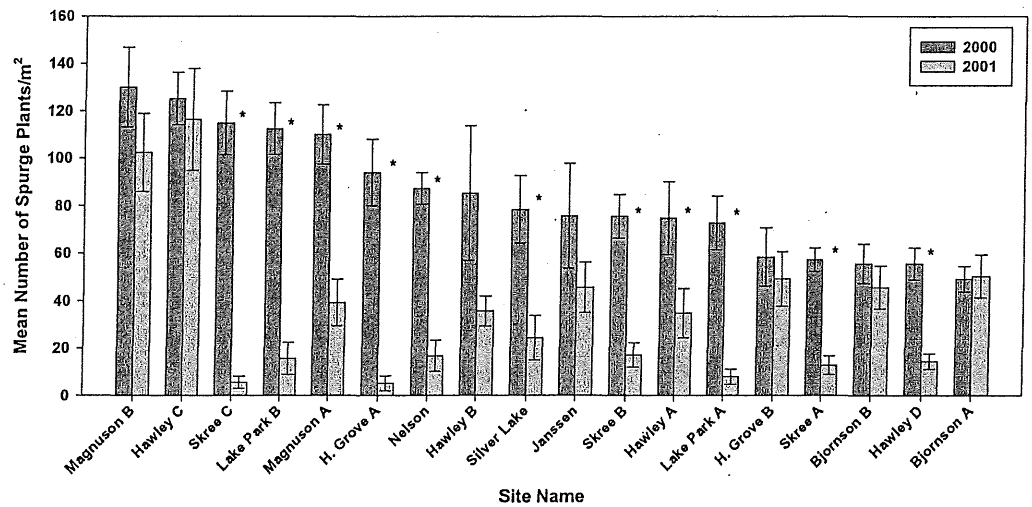


Fig.2. Mean with standard error for combined totals of flowering and non-flowering spurge plants per square meter for sites in Clay and Becker Counties.*denotes significant differences (p=0.05).



Leafy spurge density (total number, flowering and non-flowering) and percent cover were all significantly reduced in 73% of the sites (Fig.2, Fig.3). This reduction on average took three years. This is exceptionally fast for biological control to be successful. The root-feeding larvae are most likely the key, stressing or killing the plants by destroying the carbohydrate reserves in the roots (Gassman et al. 1996). Biocontrol agents that only defoliate plants tend to take longer in controlling perennial plants due to reserves in the roots, which may take two or more years of defoliation to kill the plant (Katovich et al. 1999) We would expect a greater percentage of significant decrease in spurge density in the summer 2002 than 2001.

Many of the sites were former pasture comprised of smooth brome grass. Since this grass was the predominant species prior to spurge infestation, it is not surprising that we found this species extensively in our study. As spurge density decreased, the resulting area was filled with plant species already present at the site which in this study was primarily smooth brome.

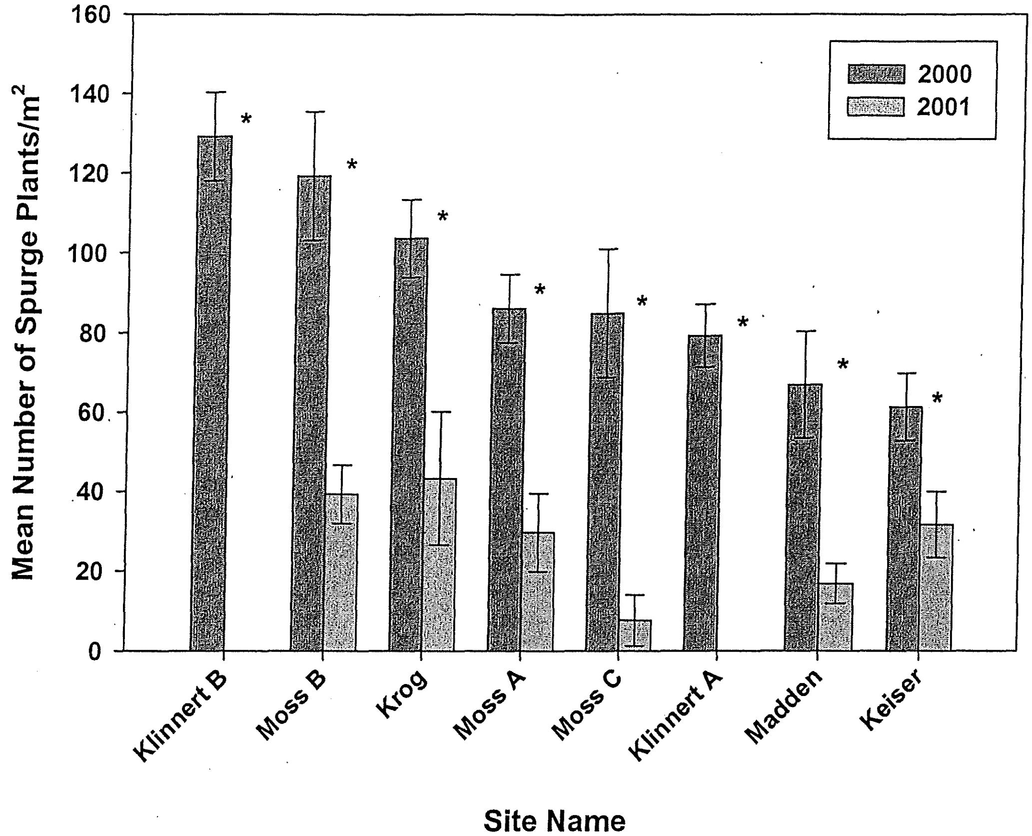


Fig. 3. Mean with standard error for combined totals of flowering and non-flowering spurge plants per square meter for sites in Otter Tail County. * denotes significant differences (p= 0.05).

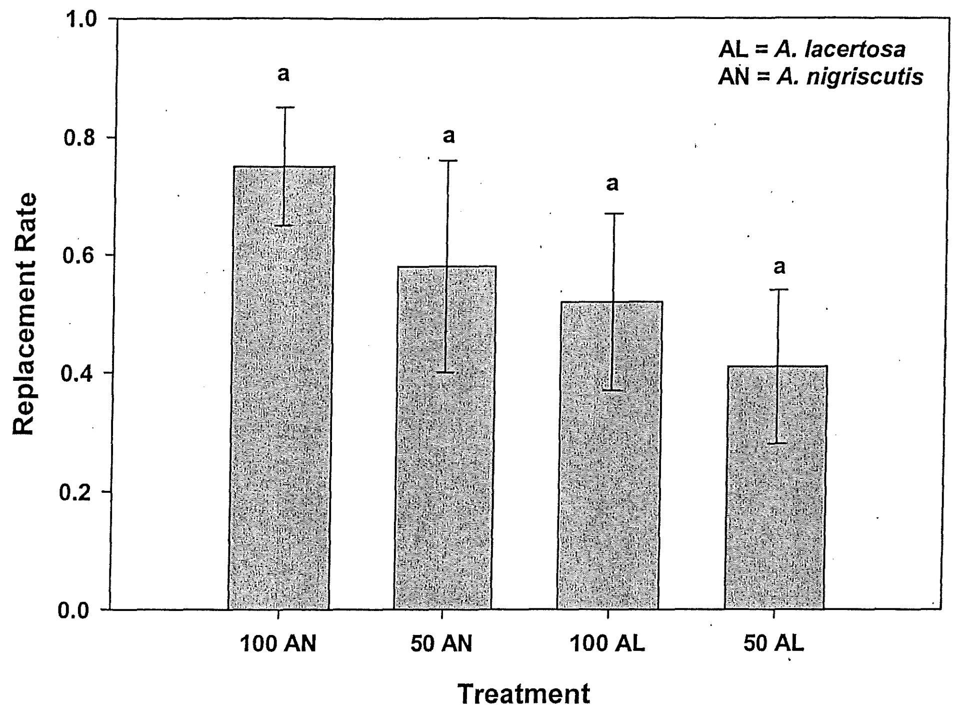
Objective 2. Test fpr interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis.*

Results:

One year post treatment, there were no significant differences between treatment replacement rates. (Figure 4). The mean replacement rate for all *Aphthona* species and treatments was 0.56. Replacement rates across all treatments ranged from 0.07 to 0.92.

Leafy spurge stem densities significantly decreased one-year post treatment for all treatments. Prior to treatment the mean leafy spurge stem density per plot was 239.6. Leafy spurge densities per plot ranged from 178 to 301 stems. Leafy spurge stem densities, however, significantly decreased one-year post treatment (Figure 4). The mean stem density per plot across all treatments was reduced to 86.4 stems, and stem density ranged from 6 to 123.

Figure 4. Replacement rates of *Aphthona* one year post treatment. There were no significant differences by treatment as indicated by letter 'a'



Objective 3. Test effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis.*
Results.

Within treatments, *A. lacertosa* established at all plots and *A. nigriscutis* was recovered from all but one plot. At peak emergence, the total number of *A. lacertosa* per plot ranged from 9 to 188 compared to *A. nigriscutis* ranging from 0 to 69 per 50 sweeps. Neither species affected the establishment of the other in the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatment.

The 1,000 *A. lacertosa* treatment produced the highest insect density that was significantly higher than all treatment except the combined 500 *A. lacertosa*/500 *A. nigriscutis* release rate ($P = 0.05$, Fig. 14). In contrast, there were no differences among the release rates of *A. nigriscutis*.

All plots initially contained spurge with a mean number of total (flowering and non-flowering stems) stems ranging from 42 to 169 stems per m^2 (Fig. 13). Density of flowering, non-flowering, or total number of spurge stems in general was not significantly different for most treatments ($P = 0.05$, Table 13). In 2001, mean number of spurge stems across all treatments ranged from 26 to 110 and 1 to 89 stems per m^2 for flowering and non-flowering spurge respectively. Significant reductions in the total number of spurge stems were observed in the 1,000 *A. nigriscutis*, 250 *A. lacertosa*, and the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatments. Spurge height ranged from 15 to 97 cm with a mean of 65.9 cm which was not different from stem height prior to release and one treatment, 250 *A. lacertosa,* showed a significant increase in stem height. Mean percent spurge cover for all plots ranged from 6-25% to 76-100%. The only significant change was an increase in spurge cover with the 1,000 *A. nigriscutis* treatment. The mean number of plant species per plot other than spurge was 1.6 species. Perennial grasses were the predominant vegetation type found in the plots followed by perennial forbs, annual or biennial forbs, and woody perennials respectively (Table 12).

Table 12. Categories of plant species other than leafy spurge found during vegetation surveys.

Category	**2000**	**2001**
annual or biennial forb	8.65%	6.85%
perennial grass	62.63%	83.06%
perennial forb	25.95%	9.68%
woody perennial	2.77%	0.40%

Objective 4. Develop a degree-day emergence model for *Aphthona* adults.
Results.

Developmental Rates and Lower Developmnental Threshold Determination:
A total of 3,355 *A. lacertosa* individuals were collected from the growth chambers while only 277 *A. nigriscutis* individuals were collected. Developmental times decreased with increasing temperatures. Average days to emergence ranged from 68.6 days at 15°C to 25.6 days at 26°C for *A. lacertosa* and 84.3 days at 15°C to 26.1 days at 26°C for *A. nigriscutis*. The development rates (1/d) for *A. lacertosa* and *A. nigriscutis* were linear with temperature as shown in Figures 15 and 16 respectively. Based on the regression analysis, the lower developmental threshold estimate for *A. lacertosa* and *A. nigriscutis* are 8.3 °C and 10.1 °C respectively. The degree-days required for adult emergence are 448 for *A. lacertosa* and 425 for *A. nigriscutis* based on the reciprocal of the slope of the linear regression.

Phenology Model

Pooled data resulted in four "location years" as described by Legg et al. (2002). Model equations and R^2 value for non-linear models are provided. The estimated accumulated degree-days to peak abundance of *A. lacertosa* is 513 (based on a lower developmental threshold of 8.3 °C). The estimated accumulated degree-days to peak abundance of *A. nigriscutis* is 610 (based on a lower developmental threshold of 10.1 °C). Calculations, estimates and standard errors for predicting the number of accumulated degree-days at peak abundance are listed.

Displaying Models Spatially
Maps displaying estimated average dates to peak abundance for *A. lacertosa* and *A. nigriscutis* are displayed in Figure 21. Average date to peak abundance for *A. lacertosa* ranged from June 17th to July 22nd depending on location in the state. Average date to peak abundance for *A. nigriscutis* ranged from June 27th to August 8th, approximately 10 days later statewide than *A. lacertosa* in similar geographic locations. Peak emergence occurred earlier in the year in the southern part of the state for both species and became progressively later as you move north and east in the state.

V. DISSEMINATION

Data will be shared with each of the Counties as well as provide statewide maps. Presentations will be made at the County Agricultural Inspectors' Workshop and at district weed meetings.
Findings will be shared with other State and Federal Agencies.

VI. CONTEXT:

A. Significance:

The five species of *Aphthona* flea beetles have been released since 1989 and all of them are established in Minnesota. However, some of them have had delayed establishment taking 3-4 years to reveal damage symptoms. This variability has prompted this investigation to analyze flea beetle establishment and habitat characteristics.

Special conditions such as unusual weather events and records of treatment history, including information on treatment applications (where, how, cost, and successes) will allow evaluation and fine-tuning of treatments. Biological control does not aim to eradicate weeds, but to keep them at low, manageable levels. The five flea beetle species have taken 5-10 years to establish and increase to effective numbers. This study is expected to provide information on action thresholds and prioritizing and balancing treatments with resources. County Weed Inspectors will be able to use this biological control tool to manage weeds in their counties in addition to or as an alternative to current control practices.

B. Time: The proposed project will exceed two fiscal years because of its seasonal field work.

C. Context: MDA enforces the Noxious Weed Law which entails surveying for noxious weeds in each county by the county Ag.Inspectors who are paid by the counties but supervised by MDA. MDA's Plant Pest Survey and Biological Control Program also interacts with the county ag.inspectors by providing biocontrol agents and assisting in their establishment and monitoring. USDA, APHIS cooperates in the redistribution of biocontrol agents from their established sites.

LCMR Project Budget Breakdown (revised)

BUDGET: (2000-2002)

Personnel: Dr. Dharma Sreenivasam, MDA. 5% in-kind contribution.
Dr. David Ragsdale, Professor, Dept. of Entomology, University of Minnesota. 5% in-kind contribution.

	Budget	Expenses
Research Associate (100%) MDA	$ 71,736	71,736.00
Research Assistant (50%) U of M	$ 50,264	50,264.00
~~**Space Rental** Office and Lab.~~	~~$ 3,000~~	
Communications, Telephone, Mail	$ 600	600.00
Contracts: Professional & Technical	$ 5,000	5,000.00
	$ 3,000	3,000.00
In-State Travel	~~$ 5,000~~	
	$ 6,000	6,000.00
Out-of-State Travel	~~$ 2,000~~	
	$ 1,000	1,000.00
Office Equipment, Supplies	$ 2,400	2,400.00
Totals	**$140,000**	**140,000.00**

VII. COOPERATION: As shown in the budget.

VIII. LOCATION: As shown in the report.

Recommendations:

We recommend that *A. lacertosa* be used as the primary agent for control of leafy spurge in Minnesota. We also recommend that *A. nigriscutis* continue to be redistributed statewide, particularly to sites where *A. lacertosa* may not do well. Mixed colonies are preferred for sites that are very dry with sandy soils. Efforts to collect and redistribute *A. cyparissae* and *A. flava* should be considered low priority, unless field populations become highly abundant.

The current practice of releasing >4,000 beetles per leafy spurge infestation, should be continued. If flea beetle abundance becomes low, smaller release quantities can be released with some success.

We recommend that release sites be visited starting two years post release. Our results show that flea beetle populations increase dramatically in the second or third year. Leafy spurge reduction, on average, also begins to decline rapidly three years post release, but may occur as early as two years post release. Importance of monitoring two years post release are two-fold, first to monitor success of the biocontrol release and secondly to determine if flea beetle populations are large enough that a collection can be made for redistribution

Document 2 – Final Report Abstract

Title: *Evaluate establishment, impact of leafy spurge biocontrol agents.*

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Leafy spurge is a Eurasian perennial plant that seriously impacts native plants, wildlife, and grazing land for cattle and horses. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge. Some of the species, however, have had difficulty establishing and have not contributed to control success. Factors that may affect insect establishment include soil type, soil moisture, leafy spurge density, leafy spurge biotype, vegetation type, litter cover, release quantity, and interspecific competition.

The results suggest that *A. lacertosa* is the most effective species in controlling leafy spurge in Minnesota. *Aphthona lacertosa* established at 100% of the release sites and significantly reduced leafy spurge by 63% across all sites studied. *Aphthona nigriscutis* established at 73% of the study sites, but at significantly lower densities than *A. lacertosa*. *Aphthona nigriscutis* most likely contributed to the control success at sites where both species occurred. Other introduced *Aphthona* species are difficult to locate in Minnesota and contributed little to the overall control success occurring statewide. Correlations between biotic/abiotic factors and flea beetle density were not clearly evident. Only soil texture seemed to affect *A. lacertosa* densities, which may not have biological significance. Early indications showed that interspecific competition between *A. lacertosa* and *A. nigriscutis* was not affecting flea beetles populations. Small release quantities (<500 beetles) may have contributed to lack of establishment on early releases made in Minnesota. Currently it is recommended that >1,000 beetles should be released at new leafy spurge infestations. Phenology models predicting peak emergence of *A. lacertosa* and *A. nigriscutis* were developed to provide information to resource managers on when to collect beetles for redistribution.

<u>Document 3-</u> Detailed Research Addendum

Evaluate Establishment, Impact of Leafy Spurge Biocontrol Agents

By

Luke C. Skinner
Monika Chandler
David Ragsdale
Dharma Sreenivasam

July 1, 2002

Funding provided by the Minnesota Environment and Natural Resources Trust Fund

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Extensive Study

Interspecific competition between *Aphthona lacertosa* and *A. nigriscutis*

Effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis*

Abstract

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Leafy spurge is a Eurasian perennial plant that seriously impacts native plants, wildlife, and grazing land for cattle and horses. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge. Some of the species, however, have had difficulty establishing and have not contributed to control success. Factors that may affect insect establishment include soil type, soil moisture, leafy spurge density, leafy spurge biotype, vegetation type, litter cover, release quantity, and interspecific competition.

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Introduction

Leafy spurge, *Euphorbia esula*, is an Eurasian perennial plant that was introduced to North America in the early 19th century (Britton 1921, Dunn 1979). It was thought to have arrived in Minnesota around 1890 with a contaminated shipment of oats from Russia (Best et al. 1980, Kommendahl and Johnson 1959). Like many exotic plants, leafy spurge's natural enemies were left behind in its native range, allowing the plant to dominate similar habitats in North America (Belcher and Wilson 1989). The superior competitive abilities (rapid growth and allelopathic properties) of leafy spurge caused the reduction of native grasses and forbs normally consumed by wildlife (Steenhagen and Zimdahl 1979, Belcher and Wilson 1989). Leafy spurge produces a milky latex that can act as an irritant causing blisters or hair loss in horses, act as a laxative and induce vomiting in many animals when eaten (Best et al. 1980, Muenscher 1935, 1960). Since wildlife and cattle generally avoid grazing in leafy spurge infested areas, carrying capacity of infested pastureland may be reduced by up to 75% (Hein and Miller 1992, Kronberg et. el. 1993, Lacey et al. 1984, and Trammel and Butler 1995).

Leafy spurge reproduces both by seed and vegetative root buds. Seed dispersal can be aided by wildlife, wind, water, and humans. Disturbance often plays a role in the spread of spurge. Belcher and Wilson (1989) determined that 95% of spurge colonies found within a mixed-grass prairie were centered on trails, fireguards, road construction, and disturbances caused by tracked vehicles turning. A patch of leafy spurge can spread vegetatively from 1 to 3 feet per year (Lym et al. 1998).

Leafy spurge is widely established in the northern half of the United States (Dunn 1979). Leafy spurge infests more than 1.6 million acres in the four-state region of Montana, North Dakota, South Dakota, and Wyoming alone (Leitch et al. 1994). It is estimated that direct and indirect impacts to grazing lands and wildlife habitat due to leafy spurge has resulted in an estimated economic loss of $130 million annually to the upper great plains (Leitch et al. 1994). Leafy spurge is difficult to control with conventional methods such as chemical, cultural and mechanical control (Lym and Messersmith 1987, 1994). These methods are very costly and usually do not provide long-term control of this plant (Lavigne 1984, Lym and Messersmith 1987, 1994).

Alternative long-term control methods, such as biological control, are considered valuable leafy spurge management tools in North America. In its native range, leafy spurge is kept in check by natural enemies, mainly insects (Gassmann and Schroeder 1995). Since 1964, ten insect species have been introduced into the United States for the control of leafy spurge. These include a plant defoliating moth, *Hyles euphorbiae*; a stem and root boring beetle, *Oberea erythrocephala*; a gall forming midge, *Spurgia esula*; a moth whose larvae attack the roots, *Chamaesphecia hungarica*; and six species of flea beetles that attack both roots and foliage, *Aphthona abdominalis, A. cyparissiae, A. czwalinae, A. flava, A. lacertosa, and A. nigriscutis* (Gassmann and Schroeder 1995, Hansen et al. 1997, Harris 1996, Rees et al. 1996, Rees and Spencer 1991, Spencer 1994). The majority of the insects have been introduced in the last decade with varying degrees of success (Hansen 1998, Hansen et al. 1997, Rees et al. 1996, Rees and Spencer 1991, Spencer 1994). Success of each control agent may depend on suitable habitat, climate or other pressures such as quality of food or predation. We will limit our scope to the flea beetles *Aphthona* spp., which are responsible for control success within Minnesota and western states.

***Aphthona* species: Life histories and potential for control**

Six *Aphthona* species of flea beetles were released in the United States to control spurge. Adult *Aphthona* spp. feed on leafy spurge foliage. Females lay small groups of eggs at, or just below, the soil surface, near the base of a leafy spurge stem. Newly hatched larvae burrow in the soil and begin feeding on very small leafy spurge roots. The larval feeding on the roots destroys the root section of the feeding site. Larvae feed on progressively larger roots and root buds as they develop. The aggregate feeding of many larvae on a root system can be lethal to the spurge plant. (Gassman et al. 1996). All *Aphthona* spp. released in North America are univoltine with the exception of *A. abdominalis*. Univoltine *Aphthona* spp. overwinter as larvae which resume feeding in the spring, then pupate in a soil cell in late spring to early summer (Gassman et al. 1996, Maw 1981).

Aphthona abdominalis (Coleoptera: Chrysomelidae), is the newest of the *Aphthona spp.* to be released (1993) in the Unites States (Hansen et al 1997, Rees et al. 1996, Spencer 1994). *Aphthona abdominalis* is very small (2 mm), with reddish-yellow head, prothorax and mesothorax; a black metathorax and abdomen; and the elytra are a semitransparent tan (Fornasari 1993). *Aphthona abdominalis* is the only multivoltine flea beetle of the six and can have up to four generations in a year (Fornasari 1993). Adults typically emerge from the soil in May and June, and appear to live for 40 to 55 days. Females lay eggs singly or in clusters on the plant near the soil surface, or in the soil near the base of a leafy spurge plant. Newly hatched larvae burrow into the soil and begin feeding on very small leafy spurge roots. Adults and larvae of the last generations of the season overwinter, which resume feeding in the spring. The overwintered larvae then pupate in a soil cell in late spring to early summer (Fornasari 1993).

Field tests indicate that *A. abdominalis* prefers moist soils, particularly in climates that have an average rainfall of 12-18 inches per year (Rees et al. 1996). High humidity, during the egg stage, plays a positive role in egg survival. *Aphthona abdominalis* has been released on only a few sites, none of which have become established (Hansen et al 1997). The failure to establish is not understood, but there has been some speculation that small release sizes and/or very cold climate affect their establishment. Due to the larva's ability to seriously damage shoots, shoot buds and roots, this species is still considered to have a good potential against leafy spurge (Rees et al. 1996).

Aphthona cyparissiae (Coleoptera: Chrysomelidae), was the second flea beetle to be released (1987) into the United States (Rees et al. 1996). *Aphthona cyparissiae* are small (3 mm) and yellowish-brown or bronze in color dorsally, and are a darker brown ventrally (LeSage and Paquin 1996). *Aphthona cyparissiae* are very similar to *A. nigriscutis* adults, but lack a dark scutellum (LeSage and Paquin 1996, Rees et al.1996). This flea beetle has only one generation per year (Gassmann et al. 1996, Maw 1981). Depending on location, adults emerge from the soil beginning in June or July, and are present for several weeks to several months (Maw 1981, Rees et al. 1996). This flea beetle is associated with open, sunny areas and dry sites with soils containing 40 to 60% sand (Maw 1981, Nowierski 1996, Rees et al. 1996). *Aphthona cyparissiae* may also prefer taller spurge plants (>20cm.) in densities of 11-12 stems per square foot. (Rees et al. 1996).

Aphthona cyparissiae populations appear to have "controlled" leafy spurge infestations at a number of western and mid-western North American locations (Hansen 1998). *Aphthona* *cyparissiae* established at 81% of all release sites in the United States and is rated as one of the best potential agents released against leafy spurge (Hansen et al. 1997, Maw 1981).

Aphthona czwalinae (Coleoptera: Chrysomelidae) was the first of two black colored flea beetles released into the United States. *Aphthona czwalinae* was first released in 1987 (Gassmann and Schroeder 1995). Adult flea beetles are small (3 mm), metallic black in color and easily confused with A. lacertosa (Gassmann and Schroeder 1995, LeSage and Paquin 1996), but *A. czwalinae* is distinguishable from A. lacertosa by its black metathoracic femur in contrast to *A. lacertosa*'s brown metathoracic femur (LeSage and Paquin 1996). Adults typically emerge from the soil from May to July, and are present for up three months.

Aphthona czwalinae prefers moist sites with high relative humidity and mesic, loamy soils (Maw 1981, Gassmann and Schroeder 1995, Rees et al. 1996). *Aphthona czwalinae* also prefers sites where leafy spurge is growing intermixed with other vegetation. This species can survive in warm and dry summers in well drained, sandy soils. *Aphthona czwalinae* does not do well in sites with compacted clay soils or in areas with high ant populations (Rees et al. 1996).

Mixed *A. czwalinae/A. lacertosa* populations have been released due to difficulty in telling the two species apart. These mixed populations have apparently "controlled" leafy spurge infestations at a number of sites in the western and mid-western U.S. *Aphthona czwalinae/A. lacertosa* established at 100% of all release sites in the United States and so far this has been a good combination for wetter sites (Hansen et al. 1997).

Aphthona flava (Coleoptera: Chrysomelidae), was first released in 1985 in the United States against leafy spurge (Gassmann and Schroeder 1995, Rees et al. 1996). *Aphthona flava* is the largest of the introduced yellowish-gold flea beetles (3.5mm) and is similar in color *to A. cyparissiae* and *A. nigriscutis* (LeSage and Paquin 1996). *Aphthona flava* does best in sunny, south facing slopes in cooler climates with 18-20 inches of annual rainfall. This species does not do well in heavily shaded areas or where soils are acidic or composed of clay. This species seems best suited to sites that are somewhat more mesic than those utilized by *A. nigriscutis* or *A. cyparissiae* Maw 1981, Rees. et al. 1996).

Aphthona flava populations appear to have successfully controlled leafy spurge infestations at several locations, but the overall "success rate" is lower than it is for *A. czwalinae/A. lacertosa* combination and *A. nigriscutis* (Hansen et al. 1997). *Aphthona flava* has established at 83% of the original release sites and has reached a "collectible" population size at 4% of the established sites (Hansen et al. 1997).

Aphthona lacertosa (Coleoptera: Chrysomelidae), first released in 1993, is fast becoming the most dominant flea beetle released to control leafy spurge (Gassmann and Schroeder 1995, Hansen et al. 1997). Adult *A. lacertosa* are small (3 mm), metallic black in color and are distinguishable by its brown metathoracic femur in contrast to *A. czwalinae's* black metathoracic femur (Gassmann and Schroeder 1995, LeSage and Paquin 1996). *Aphthona lacertosa* has a distinct association with loamy soils that are mesic to wet (Maw 1981). This species prefers sites with a well-established plant community inter-mixed with leafy spurge (Rees et al 1996).

Mixed populations of *A. lacertosa* and *A. czwalinae* are said to be responsible for major reductions in spurge populations in Montana and North Dakota (Hansen et al. 1997, Robert Richard, USDA-APHIS, personal communication). This includes one site in Valley City, North Dakota, where millions of flea beetles were collected and redistributed to other states. Field collected populations are typically dominated by *A. lacertosa.* Season long sweep net sample data collected from three sites near Valley City, North Dakota, produced 99 percent *A. lacertosa* and less than one percent other *Aphthona* species (Jordan 1999).

Aphthona nigriscutis (Coleoptera: Chrysomelidae), was first released in 1989 against leafy spurge in the United States (Gassmann and Schroeder 1996, Rees et al. 1996). *Aphthona nigriscutis* are small (3 mm) and yellowish-brown or bronze in color dorsally, and darker brown ventrally. They are distinguished from the other tan flea beetles by a dark scutellum, which the other species are lacking (LeSage and Paquin 1996).

Aphthona nigriscutis prefers dryer sites with sandy soils (less than 3% organic matter), that are found on hilltops (Maw 1981, Nowierski et al. 1996, Rees et al. 1996). *Aphthona nigriscutis* prefers sites with mixed vegetation and with leafy spurge stem densities less than 60 stems per square meter (Rees et al. 1996). *Aphthona nigriscutis* populations have significantly reduced spurge infestations in several western states, including Montana and Wyoming. One release site in Montana saw a greater than 70% reduction in leafy spurge densities over a 5500 m^2 area, while at another site in Wyoming spurge was eliminated from more than 6000 m^2 (Hansen et al. 1996, Van Vleet 1994). It is considered the most successful leafy spurge control agent to date. *Aphthona nigriscutis* has established in 84% of the sites where it has been released and has large enough populations on 34% of these sites to be considered "collectible." This species has been effective only on dry sites (Hansen et al. 1997).

History and current status of leafy spurge biological control in Minnesota

Leafy spurge has been in Minnesota since the 1890's. Over the last 100 years, leafy spurge has infested an estimated 800,000 acres statewide, with the heaviest infestations in the west and northwest counties (Cortilet, 2000). Leafy spurge is currently designated as a Noxious Weed in Minnesota, and is considered one of the top pest plants in Minnesota (Minnesota Department of Agriculture, Chapter 1505.0730). In 1989, Minnesota received its first shipment *A. cyparissiae* and *A. nigriscutis* for the control of leafy spurge (Minnesota Department of Agriculture- leafy spurge database). *Aphthona flava* was first released in Minnesota in 1992 from collections made near Bozeman, Montana (USDA-APHIS field records). Both *O. erythrocephala and S. esula* were first introduced in 1993. *Aphthona czwalinae* and *A. lacertosa* were originally released as mixed populations collected from Valley City, North Dakota in 1994 (USDA-APHIS field records).

Currently, all five species of *Aphthona* released in Minnesota are established. *Oberea erythrocephala* and *S. esula* were recovered at two sites, but populations remain very small (Cortilet 2000). As the flea beetle populations established and increased in numbers, collections were made and the beetles were redistributed to new sites. Collections were also made in North Dakota and redistributed in western Minnesota (P. Deerwood, USDA-APHIS, personal communication). By 1997, the number of flea beetles released in the state increased dramatically (Table1).

Table 1. Number of *Aphthona* spp. released for control of leafy spurge in Minnesota.
(Data summarized from MN Department of Agriculture leafy spurge database)

YEAR	No. of Releases	No. Insects Released
1989	8	4,500
1990	5	3,000
1991	5	4,000
1992	4	1,700
1993	3	2,000
1994	2	1,550
1995	13	14,250
1996	67	91,000
1997	158	1,511,153
1998	362	1,584,350
1999	439	4,650,322
2000	436	7,162,400
2001	539	6,797,870

The release numbers in Table 1 are conservative due to introductions that were not officially reported to the Minnesota Department of Agriculture (MDA). To date, there are an estimated 809 release sites in 66 counties in Minnesota (Cortilet 2002). The vast majority of the collections and subsequent releases as reported include *A. czwalinae*, *A. lacertosa* and *A. nigriscutis*. These collections, however, are dominated by *A. lacertosa*, with smaller numbers of *A. nigriscutis* and little or no *A. czwalinae* recovered.

Aphthona lacertosa populations significantly increased across the state starting in 1997 and account for the majority of leafy spurge reductions occurring around the state. Sites in Becker, Clay and Nicollet Counties have shown reductions in spurge densities. Examples include one site in Becker county (Forget Me Not Island Wildlife Management Area) where more than ten acres of leafy spurge were reduced to less than 10% of original stem densities in three years; a Clay county site (Rushfeldt Waterfowl Production Area) had more than 50 acres of spurge reduced to a few scattered plants; and an infestation in Nicollet county (Swan Lake Wildlife Management Area) saw more than 20 acres of spurge controlled by the flea beetles. (P. Deerwood, USDA-APHIS, personal communication, Paul Soler, USFWS, personal communication). Millions of flea beetles were collected from these sites over between 1997 and 2001 (Table 1).

Rationale for Research

Although some of the species of *Aphthona* flea beetles are successful control agents for leafy spurge, others have not established or contributed to control success. Factors that may affect establishment success include soil type, soil moisture, leafy spurge density, leafy spurge biotype, vegetation type, litter cover, and competitive exclusion (interspecific competition). *Aphthona nigriscutis* has been successful, but it is restricted to dry, sandy soils with low average annual rainfall. This is typical of states such as Montana, North Dakota and Wyoming, where *A. nigriscutis* has controlled populations of leafy spurge. In Minnesota however, *A. nigriscutis* has not been as successful which may be due to higher annual rainfall and more soils that are loamy with higher moisture content. *Aphthona nigriscutis* has impacted leafy spurge infestations in Minnesota but on a limited basis and it typically takes longer to achieve spurge control than other *Aphthona* species (P. Deerwood, USDA-APHIS, personal communication).

The most successful *Aphthona* species in moist, loamy sites has been the combination of *A. czwalinae* and *A. lacertosa*. It is known that *A. lacertosa* is the dominant species where it is released. Their populations tend to build up quickly, potentially affecting establishment of the other introduced *Aphthona* species, where mixed releases occur. In many of the release sites, it is unknown how well the other *Aphthona* species would establish in the absence of *A. lacertosa*.

Aphthona flava and *A. cyparissiae,* although present in the state have not contributed significantly to leafy spurge control in Minnesota. All of the flea beetles seem to favor sparsely to moderately infested areas. None of the species tend to control the areas of leafy spurge that are monocultures. If this is true, there is a need to consider more research for additional control agents to fill this gap. It could be that not enough time has passed to see the full effects of the control agents. With time and increasing *Aphthona* spp. populations, control of monotypic spurge infestations might be achieved.

To effectively utilize the variety of available biological control agents against leafy spurge, it is important to understand which biotic and abiotic factors influence the success of establishment and integration within spurge communities. Although there is some information in this area, a comprehensive look at current insect releases, establishment rates and environmental factors (both density dependent and density independent) will provide valuable information towards control efforts. Armed with this knowledge, biocontrol agents can be applied into situations where they have the greatest chance to succeed.

Objective of this research include:

1) Assess the relationships between establishment of *Aphthona* spp. and biotic/abiotic factors.
2) Test for interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis.*
3) Test the effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis*.
4) Develop phenological models for *Aphthona* spp using accumulated degree-days.

Objective 1. Assess relationships between establishment of *Aphthona* spp. and biotic/abiotic factors

Introduction

Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge. The species included *A. cyparissiae*, *A. czwalinae*, *A. flava*, *A. lacertosa*, and *A. nigriscutis*. Although some of the *Aphthona* species are successful leafy spurge control agents, others have not established or contributed to control success. Factors such as soil type, soil moisture, leafy spurge density, leafy spurge bio-type, vegetation type and litter cover may influence establishment success of the flea beetles. To effectively utilize the variety of available leafy spurge biological control agents, it is important to understand which biotic and abiotic factors influence the success of establishment and integration within spurge communities. A comprehensive look at biological control agent releases, establishment rates and environmental factors will provide valuable information toward control efforts.

Each *Aphthona* species thrives in specific environmental conditions. *Aphthona cyparissiae* is associated with open, sunny areas and dry sites with soils containing 40 to 60% sand (Maw 1981, Nowierski 1996, Rees et al. 1996) and may prefer taller spurge plants (>20 cm) in densities of 118-129 stems per square meter (Rees et al. 1996). *Aphthona czwalinae* prefers moist sites with high relative humidity and mesic, loamy soils (Maw 1981, Gassmann and Schroeder 1995, Rees et al. 1996) but can survive warm and dry summers in sandy soils. *Aphthona czwalinae* does not do well in sites with compacted clay soils or in areas with high ant populations (Rees et al. 1996) and prefers sites where leafy spurge grows intermixed with other vegetation. *Aphthona flava* does best on sunny, south facing slopes in cooler climates with 18-20 inches of annual rainfall and seems best suited to sites that are somewhat more mesic than those utilized by *A. nigriscutis* or *A. cyparissiae* (Maw 1981, Rees et al. 1996). *Aphthona flava* does not do well in heavily shaded areas or where soils are acidic or composed of clay. *Aphthona lacertosa* has a distinct association with loamy soils that are mesic to wet (Maw 1981). This species prefers sites with a well established plant community intermixed with leafy spurge (Rees et al 1996). *Aphthona nigriscutis* prefers dryer, hilltop sites with sandy soils of less than 3% organic matter (Maw 1981, Nowierski et al. 1996, Rees et al. 1996). *Aphthona nigriscutis* prefers sites with mixed vegetation and leafy spurge stem densities less than 60 stems per square meter (Rees et al. 1996).

Originally, we hoped to include all five *Aphthona* species established in Minnesota in our study. It was however, difficult to locate sites with enough *A. cyparissiae, A. czwalinae,* and *A. flava* to include in this study. Minnesota Department of Agriculture records document mixed species releases of *A. lacertosa* and *A. czwalinae* in Minnesota. After sorting through thousands of beetles from multiple sites, no *A. czwalinae* were identified. Although *A. czwalinae* may be present in these mixed populations, they are below detectable levels. We chose to select sites based on known releases of *A. lacertosa* and *A. nigriscutis*. This objective will assess factors that may affect *A. lacertosa* and *A. nigriscutis* establishment and leafy spurge control at 26 sites in Western Minnesota as an intensive study. To verify whether *Aphthona* spp. are established and are controlling spurge on a large variety of sites throughout the state, 59 additional field sites in 28 counties were surveyed once during the 2000 and/or 2001 field seasons as an extensive study. Data collected for this objective can potentially be used as a predictive measure of establishment for releases made at similar site types, and used in management decision making for future releases.

Materials and Methods - Intensive Study

Twenty-six sites were selected in three counties with 16 sites in Clay, 8 sites in Otter Tail, and 2 sites in Becker counties. Spurge areas were delineated using GPS. The sites are located in the same geographic region to limit variability in weather events and climate. The release history for each site is similar (Table 5) in that all sites had releases between 1997 and 1999 with approximately the same number of *Aphthona* released with one exception. Sites were characterized based on abiotic and biotic factors. Information on seven abiotic factors was collected and categorized for each site (Table 2).

Table 2. Site characteristic information collected from each release site sampled.

Characteristics			**Categories**	
Site Type	open field/prairie	shrub-prairie mix	wet prairie	woodland/meadow
Shade	none	slight (5-30%)	moderate (31-60%)	heavy (>60%)
Water Drainage	well drained	moderately drained	poorly drained	
Topography	flat	valley or swale	hillside	hilltop
Slope	level	slight slope	steep slope	
Slope Direction	north	south	east	west
Soil Texture	multiple categories			

Three soil cores from separate locations within each site were taken using a soil probe. The cores were homogenized and processed by the University of Minnesota Soil Testing Laboratory for a textural analysis. Soil textures were classified according to Saxton et. al. (1996).

Aphthona were sampled weekly for eight weeks during the 2000 and 2001 field seasons. Using a standard 15 inch diameter sweep net and 10 sweeps per sample, a designated number between 6 and 10 samples depending on the size of the site were taken for each site. The beetles were placed in plastic bags and frozen until they could be separated by species and counted. The relative proportion of males to females for each *Aphthona* species was determined from a subsample of up to 50 individuals from each site.

Vegetation data for each site were taken once during each 2000 and 2001 field season. Ten 0.25 m^2 samples per site were selected randomly using a square. For each sample the number of flowering spurge plants, number of non-flowering spurge plants, height of the 5 tallest spurge plants, percent cover of leafy spurge, and percent cover of all other vegetation was recorded. Percent cover was categorized as 0, 1-5, 6-25, 26-50, 51-75, and 76-100%. Vegetation other than spurge was further categorized as annual grass, perennial grass, annual or biennial forb, perennial forb, and woody perennial. The duff layer depth was measured (in centimeters) within each plot.

Multiple regression analysis was used to correlate insect abundance with site characteristics, spurge density, and other vegetation using backward elimination procedures. Vegetation characteristics as predictor variables included number of flowering and non-flowering spurge stems, spurge height, percent spurge cover, and percent cover by other vegetation categories. Only year 2000 vegetation data were used in this analysis due to declining leafy spurge densities in 2001. Site characteristics and duff layer depth included in the model are listed in Table 6. Also included in the analysis was the quantity of *Aphthona* released at each site and number of years after release to account for year effects.

Mean leafy spurge densities, heights, and percent cover were compared between 2000 and 2001 for each site using ANOVA. To assess the effects of time on *Aphthona* abundance and spurge densities, a graph was created of leafy spurge densities (y-axis 1) and relative *A. lacertosa* abundance (y-axis 2) versus number of years after release (x-axis). Relative abundance is defined as the proportional insect density for each year after release at a given site. For this graph, relative abundance was calculated for each site then averaged across sites for each year after release.

Results - Intensive Study

There were a range of habitat types and site characteristics amongst the study sites (Table 6). The study sites were dominated by habitat types classified as "open field/prairie" (85%), with the remaining 15% of the sites classified as "shrub prairie mix", "wet prairie", or "woodland/open spaces". Eighty four percent of the sites were not shaded, while the remaining were slightly or moderately shaded. A majority of the sites have moderate to well-drained soils. Nineteen percent of the sites were poorly drained and tended to stay damp or wet throughout the summer. The topography of the sites was predominantly flat or hillsides with slight slopes (Table 6) Only 12% of sites had steep slopes. Duff layer depth ranged from 0-7 cm. Soil texture varied with the majority classified as sandy loam (46%) or loam (27%) soils. Other soil textures recorded in study sites include loamy sand (15%), clay loam (8%) and silt loam (4%).

A total of 294,220 *A. lacertosa* and 26,546 *A. nigriscutis* individuals were sampled over a two year period. *Aphthona* spp. established at 92% of sites. Establishment is defined as greater than one beetle per sweep at peak emergence at least one year after initial release. The predominant species was *A. lacertosa* found at 100% of sites while *A. nigriscutis* was found at 73% of sites. Mean number of *A. lacertosa* per sweep at peak emergence ranged from 0.4 to 143.6 compared to *A. nigriscutis* ranged from 0.1 to 55.7. The ratios of *Aphthona* males to females are approximately equal (Figs. 5, 6).

The multiple linear regression analysis, in general, did not associate any biotic and abiotic factors with *A. lacertosa* and *A. nigriscutis* densities. *Aphthona lacertosa* abundance was negatively related to percent sand in the soil, which was the only predictor associated with insect abundance (Table 3). There were no factors related to *A. nigriscutis* abundance. This analysis includes the number of insects originally released and years since release.

Table 3. Regresion model predicting *Aphthona lacertosa* density[a]

Regressor Variable	Estimate	P value
Intercept		0.0002
Percent Sand	-0.95724	0.0122

[a]$P=0.05$, $R^2= 0.25$

The mean leafy spurge density (total number, flowering and non-flowering) across all sites in 2000 and 2001 was 21.5 stems/m^2 and 7.8 stem/m^2 respectively. Spurge density significantly decreased at 73% of sites ($p = 0.05$, Table 7, Fig. 2,3). The mean percent decrease in spurge density between 2000 and 2001 for all sites is 62.5%. The mean number of flowering spurge plants ranged from 0 to 19.4 compared to the mean number of non-flowering spurge plants ranged from 0 to 23.1 across all sites. There were significant decreases in the number of flowering spurge plants at 69% of the sites compared to significant decreases in the number of non-flowering spurge plants at 54% ($p=0.05$, Table 7). Spurge height ranged from 10 to 110 cm. Mean spurge height significantly decreased at 63% of sites ($p=0.05$). Mean percent spurge cover in 2000 and 2001 ranged from 2.3 to 4.1 and 0.0 to 4.0 respectively and decreased at 73% of sites. The number of plant species other than spurge ranged from 1 to 7 species. Perennial grasses were the predominant vegetation type found in the sites followed by perennial forbs, annual or biennial forbs, woody perennials, and annual grasses respectively (Table 4). A list of plant species recorded is shown in the appendix.

Table 4. Categories of plant species other than leafy spurge found during vegetation surveys.

Category	**2000**	**2001**
annual grass	0%	1.72%
annual or biennial forb	16.02%	12.99%
perennial grass	63.24%	63.69%
perennial forb	17.20%	19.09%
woody perennial	3.54%	2.50%

Discussion - Intensive Study

Despite a range of microclimates, we were not able to associate *Aphthona* spp. abundance with biotic or abiotic factors with one exception. It was reported that soil texture influences *Aphthona* spp. abundance (Rees et al. 1996, Lym 1998 and Nowierski et al. 2002). Our study shows that *Aphthona lacertosa* abundance may be negatively affected by soils with high sand content. Nowierski et al. (2002) suggested that *A. lacertosa* in Europe are associated with sites containing higher levels of silt and clay. We found no relationship between *A. nigriscutis* and soil texture. This is in contrast to previous research that shows an apparent relationship of *A. nigriscutis* to sandy soils and xeric conditions (Rees et al. 1996, Lym 1998, and Nowierski et al. 2002).

The lack of association with biotic and abiotic factors may suggest that *A. lacertosa* may be able to establish on a wide variety of site types. This seems to be the case in our study where *A. lacertosa* established in 100% of the sites with a wide range of population densities. This is supported by previous research that suggested *A. lacertosa* can establish in hydric, mesic, and moderately dry sites (Fornasari 1996, Gassmann 1996, Rees et al. 1996, and Nowierski et al. 2002). For *A. nigriscutis*, we cannot make this same assumption. *Aphthona nigriscutis* established in 73% of the sites but the numbers were very small and are only a fraction of *A. lacertosa* densities. The extreme low densities of all but one of the *A. nigriscutis* populations most likely affected the regression analysis. It is our impression from field observations however, that *A. nigriscutis* prefers the drier sandy sites. Rees et al. (1996) and Nowierski et al. (2002) both suggest this pattern of *A. nigriscutis* preferring drier sites with sandier soils.

The sex ratio for *A. lacertosa* and *A. nigriscutis* was approximately 50/50. This is of particular interest for *A. nigriscutis* because sex ratios for this species in western states were found to strongly favor females. It is thought that a bacteria, *Wolbachia* spp., is lethal to male *A. nigriscutis* and thus may be a barrier to establishment (D. Kazmer pers.comm.). It would be of interest to know if *A. nigriscutis* populations in Minnesota are infected with *Wolbachia* spp.

Aphthona lacertosa and/or *A. nigriscutis* have shown that they can reduce leafy spurge densities. *Aphthona lacertosa* density appears to follow a classical biological control model where the population is small shortly after release, increases exponentially, peaks then decreases as the food source (spurge) decreases. *Aphthona lacertosa* populations tended to increase then peak shortly after spurge density began to decrease (Fig. 4). For many sites, the maximum *A. lacertosa* density was reached in 2000 then dropped in 2001. There were insufficient numbers of *A. nigriscutis* to discern general trends.

Leafy spurge density (total number, flowering and non-flowering) and percent cover were all significantly reduced in 73% of the sites (Table 7). This reduction on average took three years. This is exceptionally fast for biological control to be successful. The root-feeding larvae are most likely the key, stressing or killing the plants by destroying the carbohydrate reserves in the roots (Gassman et al. 1996). Biocontrol agents that only defoliate plants tend to take longer in controlling perennial plants due to reserves in the roots, which may take two or more years of defoliation to kill the plant (Katovich et al. 1999) We would expect a greater percentage of significant decrease in spurge density in the summer 2002 than 2001.

Many of the sites were former pasture comprised of smooth brome grass. Since this grass was the predominant species prior to spurge infestation, it is not surprising that we found this species extensively in our study. As spurge density decreased, the resulting area was filled with plant species already present at the site which in this study was primarily smooth brome.

Table 5. *Aphthona* spp. release history for each site

County	Site	Date	Quantity	Species*
Becker	Lake Park A	07/97	3,000	*A. lacertosa*
		06/98	5,000	*A. lacertosa*
		06/99	10,000	*A. lacertosa/A. nigriscutis*
Becker	Lake Park B	06/99	10,000	*A. lacertosa/A. nigriscutis*
Clay	Bjornson A	06/99	12,000	*A. lacertosa*
Clay	Bjornson B	06/99	12,000	*A. lacertosa*
Clay	Hawley A	06/98	7,000	*A. lacertosa*
		06/99	12,000	*A. lacertosa*
Clay	Hawley B	06/98	10,500	*A. lacertosa*
Clay	Hawley C	06/98	4,000	*A. lacertosa/A. nigriscutis*
Clay	Hawley D	06/99	10,000	*A. lacertosa*
Clay	Highland Grove A	06/98	3,000	*A. lacertosa*
Clay	Highland Grove B	06/98	5,000	*A. lacertosa*
Clay	Janssen	06/99	12,000	*A. lacertosa*
Clay	Magnuson A	06/98	56,000	*A. lacertosa*
Clay	Magnuson B	06/98	10,000	*A. lacertosa*
Clay	Nelson	06/97	9,000	*A. lacertosa*
		06/98	5,000	*A. nigriscutis*
Clay	Silver Lake	06/98	2,500	*A. nigriscutis*
Clay	Skree A	06/98	18,500	*A. lacertosa*
Clay	Skree B	06/97	10,000	*A. lacertosa*
Clay	Skree C	06/98	8,500	*A. lacertosa*
Otter Tail	Keiser	06/99	10,000	*A. lacertosa*
Otter Tail	Klinnert A	06/98	3,000	*A. lacertosa*
Otter Tail	Klinnert B	06/98	5,000	*A. lacertosa*
Otter Tail	Krog	06/99	10,000	*A. lacertosa*
Otter Tail	Madden	07/99	10,000	*A. lacertosa*
Otter Tail	Moss A	06/98	7,000	*A. lacertosa/A. nigriscutis*
Otter Tail	Moss B	06/98	6,000	*A. lacertosa/A. nigriscutis*
Otter Tail	Moss C	06/98	7,000	*A. lacertosa/A. nigriscutis*

* Species indicates predominant species released. Small quantities of other species may have been present at release.

Table 6. Site characteristics

County	Site Name	Habitat Type	Shade	Duff (cm)	Drainage	Topography	Slope	Slope Direction	Soil Texture
Becker	Lake Park A	open field/prairie	none	2.5	well drained	hillside	steep slope	south	clay loam
Becker	Lake Park B	open field/prairie	none	3.0	well drained	hillside	steep slope	west	clay loam
Clay	Bjornson A	open field/prairie	none	2.0	moderately drained	flat	level		loamy sand
Clay	Bjornson B	open field/prairie	none	2.0	moderately drained	flat	level		loamy sand
Clay	Hawley A	open field/prairie	none	0.0	well drained	flat	slight slope	east	sandy loam
Clay	Hawley B	open field/prairie	none	0.0	moderately drained	valley or swale	level		sandy loam
Clay	Hawley C	open field/prairie	none	0.0	moderately drained	hillside	slight slope	south	loamy sand
Clay	Hawley D	open field/prairie	none	2.5	well drained	flat	level		sandy loam
Clay	Highland Grove A	wet prairie	none	0.0	poorly drained	flat	level		loam
Clay	Highland Grove B	wet prairie	none	0.0	poorly drained	flat	level		loam
Clay	Janssen	open field/prairie	none	4.0	poorly drained	flat	level		loam
Clay	Magnuson A	shrub-prairie mix	slight	7.0	poorly drained	flat	level		sandy loam
Clay	Magnuson B	open field/prairie	none	5.0	poorly drained	flat	level		sandy loam
Clay	Nelson	open field/prairie	none	1.0	well drained	hillside	slight slope	east	loam
Clay	Silver Lake	open field/prairie	none	3.0	well drained	hillside	slight slope	east	sandy loam
Clay	Skree A	open field/prairie	none	5.0	well drained	hillside	slight slope	south	silt loam
Clay	Skree B	open field/prairie	none	0.0	well drained	hillside	slight slope	west	sandy loam
Clay	Skree C	open field/prairie	none	3.0	moderately drained	hillside	slight slope	north	loam
Otter Tail	Keiser	open field/prairie	none	1.0	well drained	flat	level		sandy loam
Otter Tail	Klinnert A	woodland/meadow	moderate	1.5	well drained	hillside	slight slope	northwest	loam
Otter Tail	Klinnert B	open field/prairie	slight	2.5	well drained	hillside	steep slope	east	sandy loam
Otter Tail	Krog	open field/prairie	moderate	4.0	well drained	hillside	slight slope	south	loam
Otter Tail	Madden	open field/prairie	none	1.0	well drained	hillside	slight slope	east	sandy loam
Otter Tail	Moss A	open field/prairie	none	3.5	well drained	hillside	slight slope	west	sandy loam
Otter Tail	Moss B	open field/prairie	none	3.5	well drained	hillside	slight slope	south	loamy sand
Otter Tail	Moss C	open field/prairie	none	3.5	well drained	hilltop	slight slope	northeast	sandy loam

Table 7. Comparison of mean number of spurge plants per m^2 with standard error by year. Bold indicates a significant difference (p=0.05) between years.

County	Site Name	Number Flowering Spurge 2000	Number Flowering Spurge 2001	p	Number Non-Flowering Spurge 2000	Number Non-Flowering Spurge 2001	p	Total Number Spurge 2000	Total Number Spurge 2001	p
Becker	Lake Park A	41.2±7.93	0.4±0.40	**0.000**	31.6±10.40	7.6±3.29	**0.041**	72.8±11.31	8.0±3.21	**0.000**
Becker	Lake Park B	49.6±8.03	1.6±0.88	**0.000**	62.8±8.56	14.0±6.57	**0.000**	112.4±10.95	15.6±6.81	**0.000**
Clay	Bjornson A	28.8±5.90	22.8±3.68	0.399	20.4±5.70	27.6±6.97	0.434	49.2±5.33	50.4±9.07	0.910
Clay	Bjornson B	44.0±6.39	34.8±7.96	0.379	11.6±4.15	10.8±2.86	0.876	55.6±8.29	45.6±9.05	0.426
Clay	Hawley A	29.2±8.26	16.8±5.16	0.219	45.6±10.90	18.0±5.85	**0.039**	74.8±15.32	34.8±10.43	**0.045**
Clay	Hawley B	30.8±6.67	17.2±4.09	0.099	54.4±25.90	18.4±4.55	0.188	85.2±28.33	35.6±6.35	0.105
Clay	Hawley C	32.8±6.74	38.8±10.67	0.640	92.4±11.27	77.6±13.65	0.414	125.2±11.06	116.4±21.51	0.720
Clay	Hawley D	21.2±2.15	7.6±2.27	**0.000**	34.4±5.50	6.8±1.98	**0.000**	55.6±6.73	14.4±3.28	**0.000**
Clay	Highland Grove A	21.6±6.96	1.2±1.20	**0.010**	72.4±13.73	4.0±2.53	**0.000**	94.0±14.00	5.2±3.16	**0.000**
Clay	Highland Grove B	23.6±5.80	22.4±7.57	0.901	34.8±7.71	26.8±6.88	0.449	58.4±12.28	49.2±11.50	0.591
Clay	Janssen	26.0±6.43	20.4±4.56	0.486	49.6±18.63	25.2±7.33	0.239	75.6±22.03	45.6±10.67	0.236
Clay	Magnuson A	56.8±7.47	13.6±5.03	**0.000**	53.2±9.26	25.6±6.00	**0.022**	110.0±12.64	39.2±9.88	**0.000**
Clay	Magnuson B	64.4±16.14	51.6±8.53	0.492	65.6±15.17	50.8±8.89	0.411	130.0±16.85	102.4±16.43	0.256
Clay	Nelson	37.6±5.60	5.6±2.81	**0.000**	49.6±6.34	11.2±5.36	**0.000**	87.2±6.74	16.8±6.58	**0.000**
Clay	Silver Lake	23.2±4.65	5.6±1.71	**0.002**	55.2±10.12	18.8±8.64	**0.014**	78.4±14.26	24.4±9.36	**0.005**
Clay	Skree A	36.8±2.59	0.4±0.40	**0.000**	20.4±3.93	12.4±3.98	0.170	57.2±4.95	12.8±3.90	**0.000**
Clay	Skree B	40.0±7.16	10.8±3.82	**0.002**	35.6±7.43	6.4±1.90	**0.001**	75.6±9.17	17.2±5.10	**0.000**
Clay	Skree C	60.0±8.13	0.4±0.40	**0.000**	54.8±9.28	5.2±2.67	**0.000**	114.8±13.49	5.6±2.61	**0.000**
Otter Tail	Keiser	45.6±8.00	17.2±4.99	**0.008**	15.6±2.49	14.4±5.60	0.847	61.2±8.48	31.6±8.29	**0.022**
Otter Tail	Klinnert A	45.6±8.37	0.0±0.00	**0.000**	33.6±5.57	0.0±0.00	**0.000**	79.2±7.88	0.0±0.00	**0.000**
Otter Tail	Klinnert B	45.6±9.58	0.0±0.00	**0.000**	83.6±11.27	0.0±0.00	**0.000**	129.2±11.17	0.0±0.00	**0.000**
Otter Tail	Krog	54.4±7.23	12.8±5.33	**0.000**	49.2±8.76	30.4±11.55	0.211	103.6±9.72	43.2±16.74	**0.006**
Otter Tail	Madden	38.4±8.75	7.2±2.91	**0.003**	28.4±6.76	9.6±4.22	**0.030**	66.8±13.44	16.8±5.05	**0.003**
Otter Tail	Moss A	53.2±8.02	15.6±5.58	**0.001**	32.8±5.02	20.0±4.81	0.082	86.0±8.58	29.6±9.78	**0.000**
Otter Tail	Moss B	77.6±15.24	6.8±3.21	**0.000**	41.6±9.41	32.4±7.60	0.457	119.2±16.16	39.2±7.35	**0.000**
Otter Tail	Moss C	34.8±5.06	0.0±0.00	**0.000**	50.0±15.71	7.6±6.38	**0.022**	84.8±16.12	7.6±6.38	**0.000**

Table 8. *Aphthona lacertosa* and *A. nigriscutis* sampling means and totals by year

		A. lacertosa				***A. nigriscutis***			
		Mean/Sweep		**Total Collected**		**Mean/Sweep**		**Total Collected**	
County	**Site**	**2000**	**2001**	**2000**	**2001**	**2000**	**2001**	**2000**	**2001**
Becker	Lake Park A	96.8	22.6	11,199	2,829	2.9	1.1	421	155
Becker	Lake Park B	20.0	51.5	7,034	18,439	17.3	55.7	4,127	13,684
Clay	Bjornson A	5.8	0.9	769	202	0.4	0.7	55	71
Clay	Bjornson B	1.8	1.5	161	156	0.2	0.2	18	20
Clay	Hawley A	17.3	7.4	6,823	2,818	1.0	2.3	293	503
Clay	Hawley B	32.6	19.7	9,952	3,398	0.8	1.9	229	374
Clay	Hawley C	0.6	8.4	219	2,737	1.3	7.9	332	2,860
Clay	Hawley D	3.1	40.4	587	4,623	0.4	0.4	36	31
Clay	Highland Grove A	17.4	20.2	3,409	3,553	1.8	2.0	241	162
Clay	Highland Grove B	2.2	12.1	481	2,836	0.1	0.4	18	70
Clay	Janssen	3.7	2.7	371	454	0.1	0.1	6	9
Clay	Magnuson A	24.7	28.1	8,412	11,753	0.1	0.0	9	6
Clay	Magnuson B	2.8	21.8	867	3,344	0.0	0.0	8	5
Clay	Nelson	35.8	5.6	5,357	1,092	1.4	0.9	327	221
Clay	Silver Lake	0.0	0.4	2	39	2.2	5.5	382	914
Clay	Skree A	121.8	10.0	28,225	3,340	0.1	0.1	35	10
Clay	Skree B	13.5	26.6	2,033	4,308	2.5	4.2	319	421
Clay	Skree C	42.9	71.7	22,685	10,125	0.3	0.1	68	16
Otter Tail	Keiser	4.2	4.0	587	676	0.0	0.0	2	0
Otter Tail	Klinnert A	131.6	5.8	19,195	1,251	0.0	0.0	6	3
Otter Tail	Klinnert B	143.6	32.6	24,864	6,232	0.2	0.0	27	1
Otter Tail	Krog	15.9	67.2	3,115	8,111	0.0	0.0	2	0
Otter Tail	Madden	4.2	14.5	800	3,419	0.0	0.0	1	6
Otter Tail	Moss A	29.5	33.3	5,221	6,509	0.0	0.0	1	0
Otter Tail	Moss B	40.3	38.5	4,689	5,943	0.3	0.1	37	4
Otter Tail	Moss C	41.4	101.5	8,778	10,198	0.0	0.0	0	0

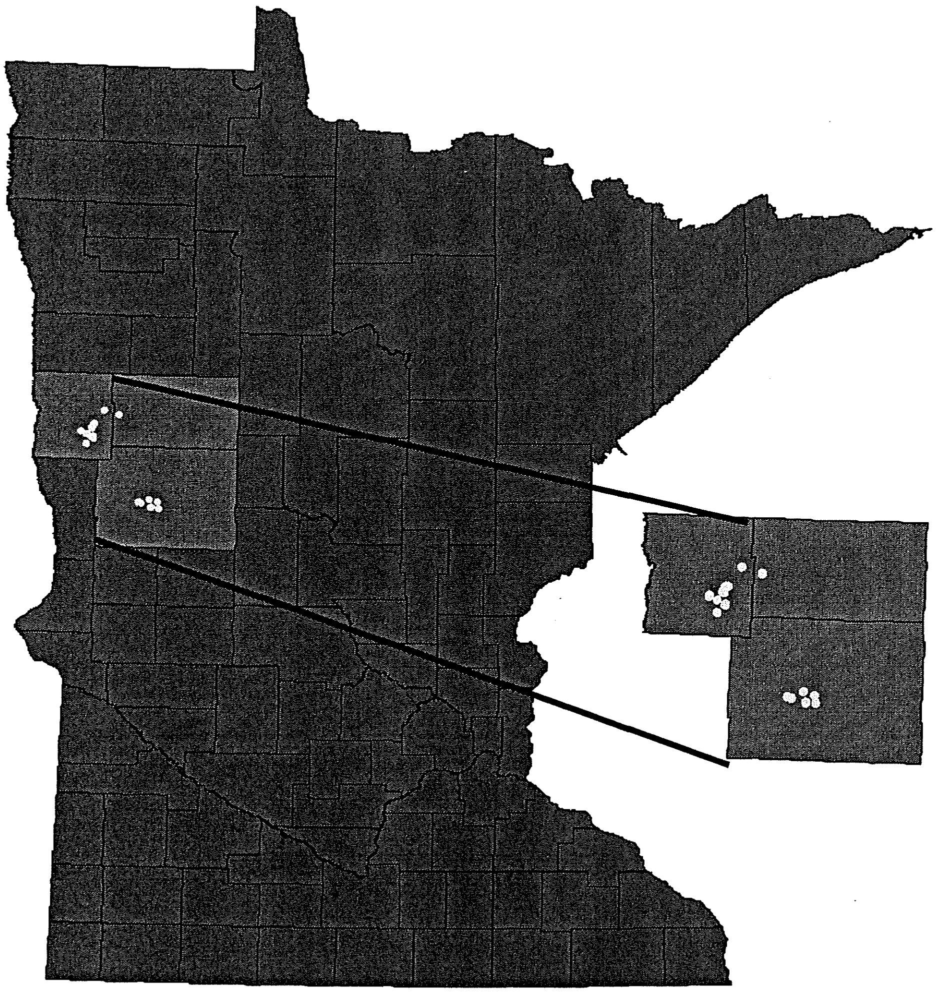

Figure 1. Locations of research sites in Northwest and West Central Minnesota

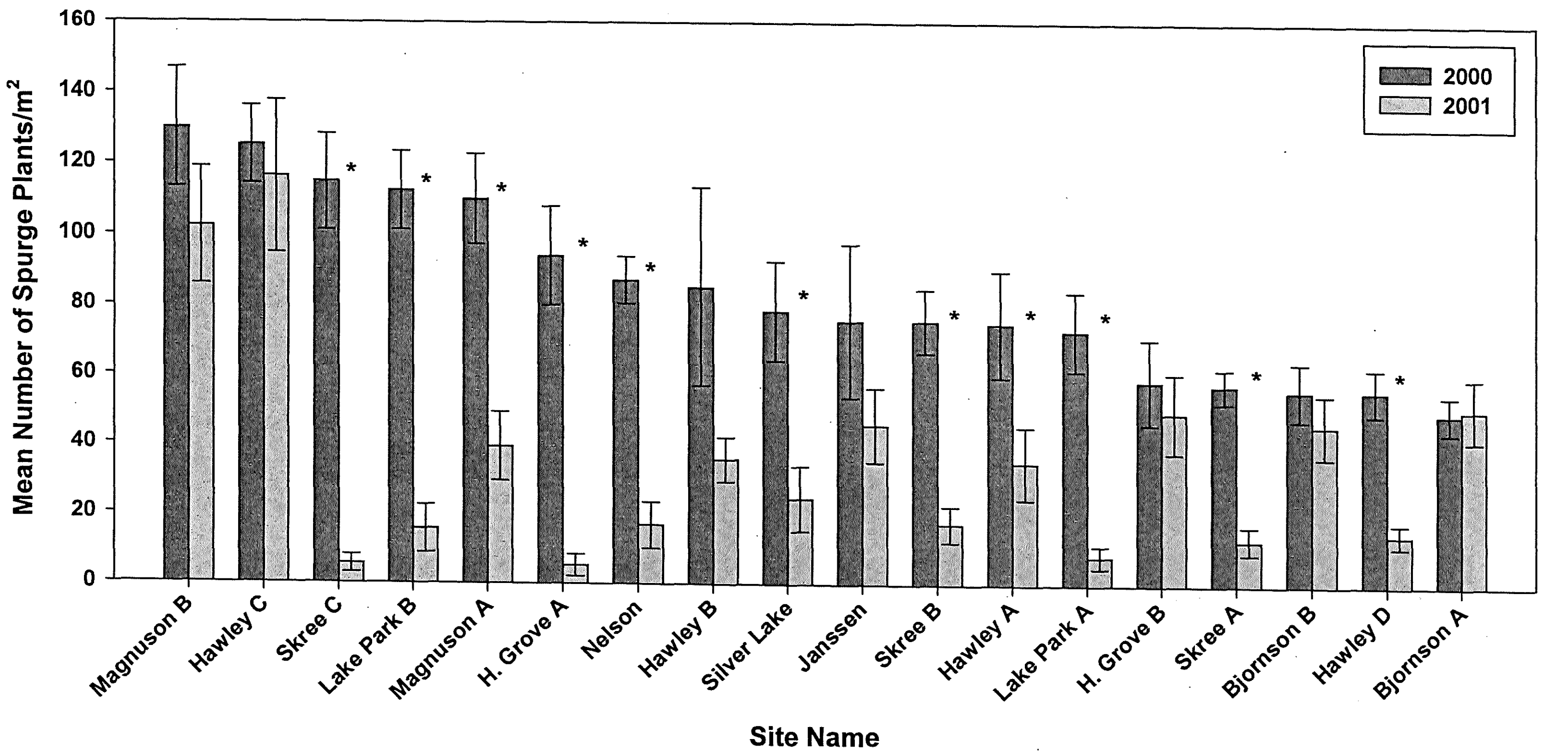

Figure 2. Mean with standard error for combined totals of flowering and non-flowering spurge plants per m^2 for sites in Clay and Becker Counties. * denotes significant differences (p=0.05).

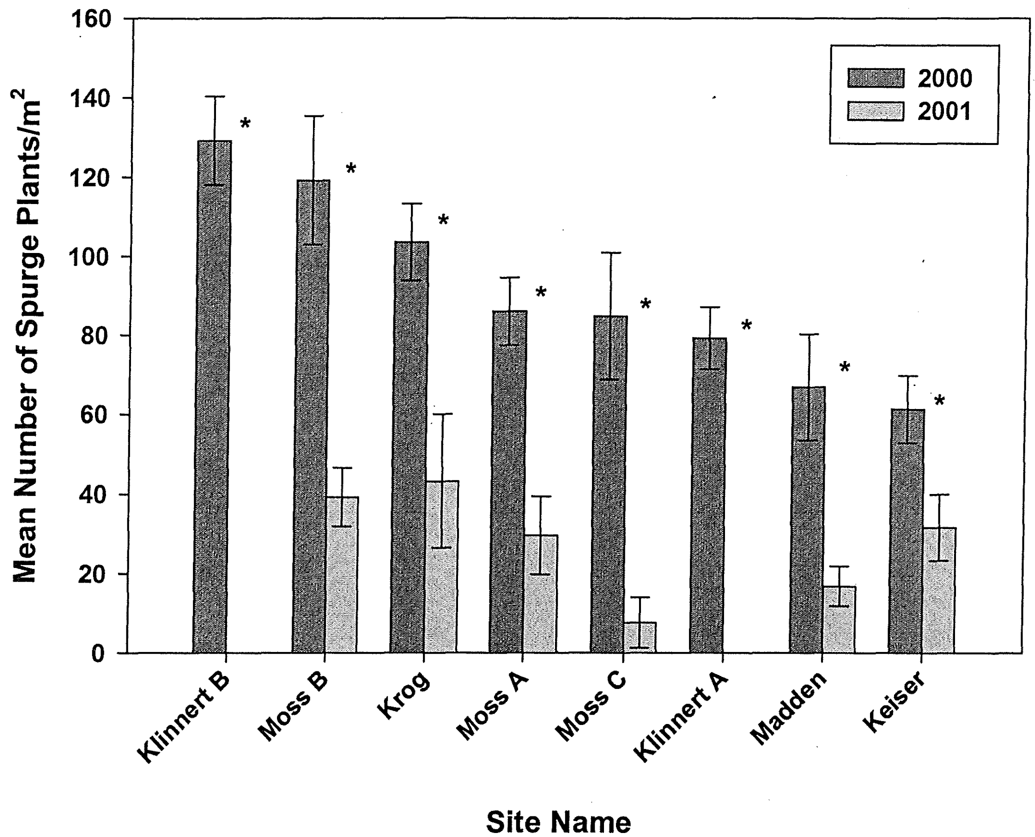

Figure 3. Mean with standard error for combined totals of flowering and non-flowering spurge plants per m^2 for sites in Otter Tail County. * denotes significant differences (p=0.05).

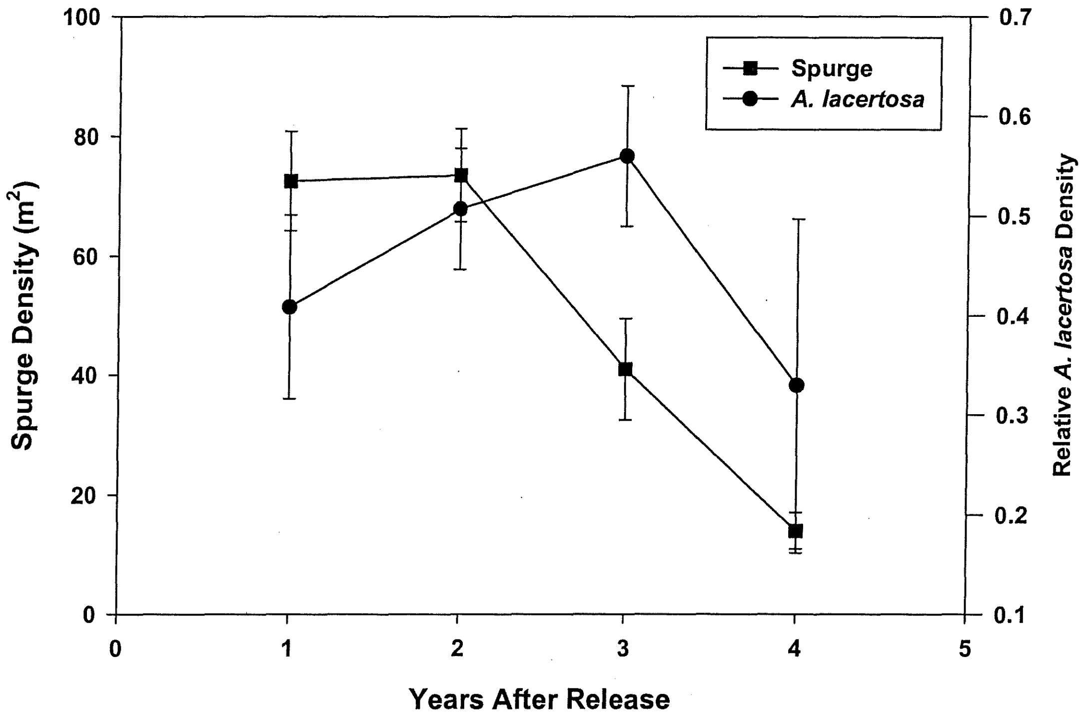

Figure 4. Mean spurge density for sites by number of years after *A. lacertosa* release compared to the relative *A. lacertosa* density by number of years after release. The relative *A. lacertosa* density is a ratio of the mean number of *A. lacertosa* per sweep by year.

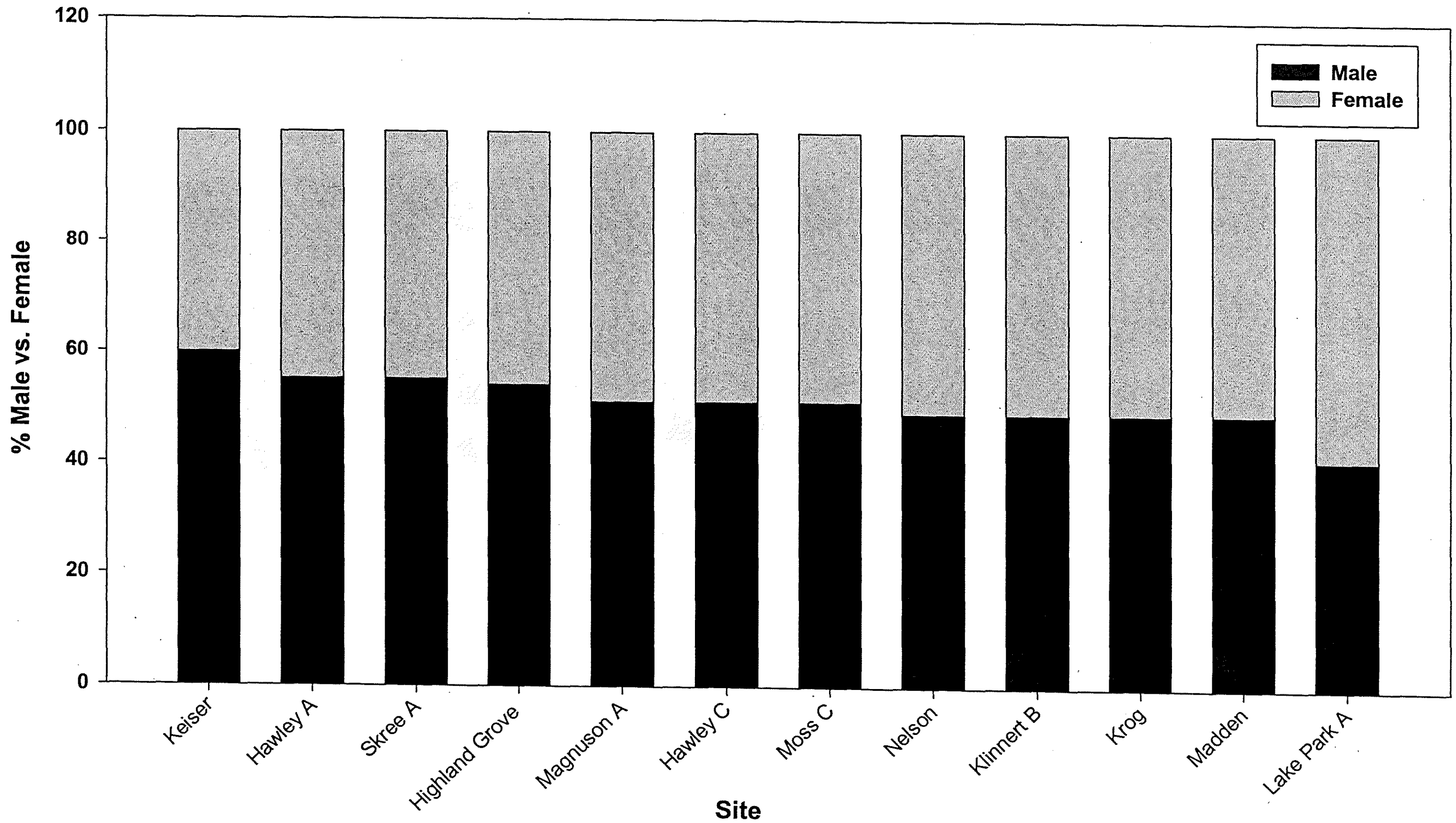

Figure 5. The percentage of *Aphthona lacertosa* males compared to females.

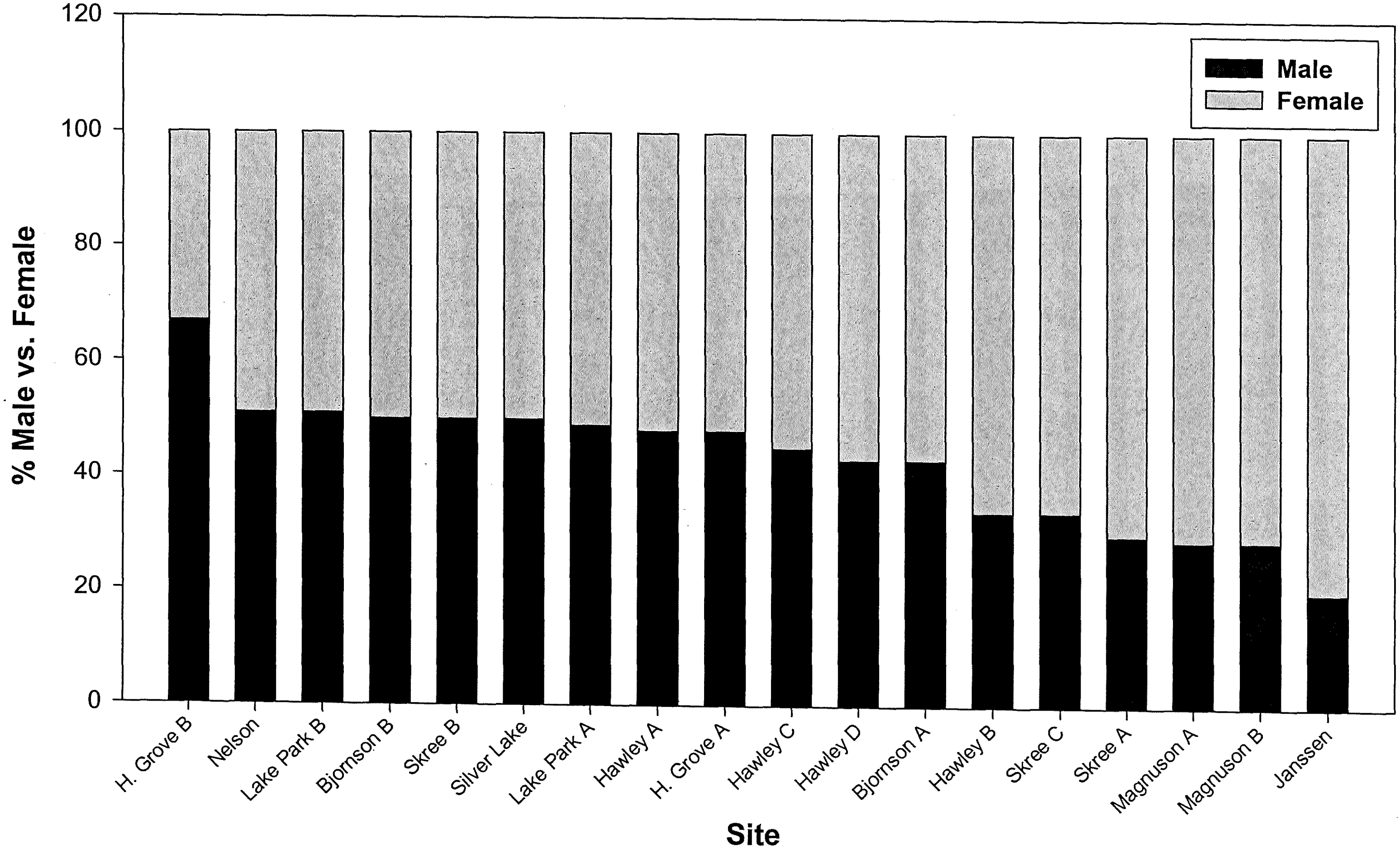

Figure 6. The percentage of *Aphthona nigriscutis* males compared to female

Materials and Methods - Extensive Study

To determine whether *Aphthona* established and is controlling spurge on a large variety of sites throughout the state, 59 additional field sites in 28 counties were surveyed once during the 2000 and/or 2001 field seasons. *Aphthona* species presence, site area, spurge area, and controlled spurge area were recorded. Site characteristics, duff layer measurements, three 0.18 m^2 samples of vegetation data, and soil samples were taken as described for the intensive study.

Results - Extensive Study

Aphthona spp. established and reduced leafy spurge in 97% of sites. The one time survey showed *A. lacertosa* establishment in 97% and *A. nigriscutis* in 29% of sites. *Aphthona flava* was recovered in small numbers from 7% of sites. *Aphthona czwalinae* and *A. cyparissiae* were not recovered from any sites. Native flea beetles, *Glyptina* spp., were found on spurge at 22% of sites.

Most of the sites were classified as "open field/prairie" (64%), followed by "shrub-prairie mix" (12%), "wet prairie" (12%), and "woodland/open spaces" (8%) respectively (Table 10). Most sites had no shade (61%) or slight shade (36%) while only 3% of sites had moderate shade. Sixty-three percent of sites were well drained, 27% of sites were moderately well drained, and 10% were poorly drained. Fifty-four percent of sites had flat topography, 34% were on a hillside, 8% were on a hilltop, and 3% were in a valley or swale. The depth of the duff layer ranged from 0 to 4 cm. Soil texture was categorized as sandy loam (37%), loam (20%), loamy sand (15%), silt loam (12%), silty clay loam (8%) clay loam (5%), and sandy clay loam (2%).

The mean number of flowering spurge plants ranged from 0.0 to 12.0 compared to the mean number of non-flowering spurge plants ranged from 0.0 to 42.7. Spurge height ranged from 3 to 94 cm. Mean percent spurge cover ranged from 0% to 76-100% across all sites. The number of plant species other than spurge found ranged from 1 to 6. Perennial grasses were the predominant species category found followed by perennial forbs, woody perennials, annual or biennial forbs, and annual grasses respectively (Table 9). A list of plant species recorded appears in the appendix.

Table 9. Categories of plant species other than leafy spurge found during vegetation surveys.

Category	**2000**	**2001**
annual grass	0.00%	0.24%
annual or biennial forb	7.06%	7.82%
perennial grass	55.88%	58.19%
perennial forb	25.29%	24.69%
woody perennial	11.76%	9.05%

The one time spurge survey data did not allow comparison to previous years as baseline data was not available. To gain a picture of *Aphthona* spurge reduction, current spurge density was plotted against number of years post release for all established sites (Fig. 8). Spurge density did decline over time. Areas of spurge control represented approximately 47% of the total site area.

Discussion - Extensive Study

Aphthona spp. established under a wide range of conditions throughout the state (Table 10). There are some sites in North Dakota where there has been difficulty establishing *Aphthona* spp. (Lym et al. 1999). We did not find any regions unsuitable for *Aphthona* spp. based on site characteristics, but we did find that sites that frequently flood can be difficult for *Aphthona* spp. establishment. The Prairie Island sites located on the banks of the Mississippi River in Winona were flooded for several weeks during the early spring of 2000 and 2001. It is possible the *Aphthona* larvae dwelling in the soil could not survive the anoxic conditions resulting from flooding. These are the only sites where *Aphthona* did not establish at all. *Aphthona lacertosa* could not be recovered after a couple of years at two very sandy sites (Grondahl turkey pen in Wadena County and Pinske in Norman County) however *A. nigriscutis* was recovered. These findings support the earlier conclusion that high levels of sand in the soil may negatively affect *A. lacertosa* establishment.

Aphthona lacertosa was the predominant species established in Minnesota (Table 11) therefore most likely to be collected and redistributed to future release sites. It appears to be a highly prolific and effective species as a biological control agent. *Aphthona nigriscutis* often appears in conjunction with *A. lacertosa*. *Aphthona flava* was released in low numbers at only a few sites. Only low quantities of *A. flava* are recovered so it is difficult to determine the effectiveness of *A. flava*, but clearly the species did not reproduce prolifically at these sites. *Aphthona czwalinae* and *A. cyparissiae* were not recovered from any sites indicating they did not establish at all in Minnesota. There were no sole releases of either *A. czwalinae* or *A. cyparissiae*, rather they were released in conjunction with other species so the initial release quantities of these species are unknown (Table 11). The native flea beetle, *Glyptina* spp., was found. There has been interest in using a native insect for spurge control, but *Glyptina* spp. is not considered sufficiently effective (Hansen 1997).

The ranges for number of flowering spurge, spurge height, percent cover and number of plant species other than spurge were similar to the ranges found in the intensive study. There were three sites at Weiler WPA in Stevens County with a much greater number of non-flowering spurge. The land manager of this site deliberately attempted to deplete the spurge seedbank by burning the site prior to *A. lacertosa* release. It appeared that burning stimulated spurge seedling germination.

Figure 8 shows spurge density declining at sites throughout the state. Spurge acreage and release quantity can influence the time required for spurge control. Another factor that can contribute to spurge density decline for a given site is immigration of *Aphthona* species not originally released. For example, *A. lacertosa* was recovered from six sites at Flandrau State Park in Brown County although this species was originally released at only three sites (Table 11). Jonsen et al. (2001) measured that *Aphthona* spp. moved up to 200 meters from an original release within two weeks. Immigration of beetles makes it difficult to attribute spurge density decline solely to the species and quantity released at a site with other proximate releases.

Plant competition may aid *Aphthona* in spurge reduction. The leafy spurge at Lake Bronson State Park site in Kittson County was growing in a restored prairie area featuring big bluestem. As the big bluestem established, it began to crowd and shade the spurge. The competition offered by the big bluestem may have contributed to the spurge decrease. Most leafy spurge sites throughout the state have non-native pasture grasses such as smooth brome. It would be interesting to examine the competitiveness of native grasses compared to leafy spurge.

There are a wide variety of practices land mangers are using in an attempt to increase the effectiveness and speed with which *Aphthona* controls spurge. As mentioned previously, one manager used burning to deplete the spurge seedbank, another manager staggered *Aphthona* releases of 10,000 beetles each in a line throughout a large field rather than one large, single release and achieved very good spurge control quickly, and other managers tried to release only beetles collected in a proximate geographic area so the beetle life-cycle stage is synchronized with spurge flowering. The study of whether such practices facilitate spurge control could have highly practical results. The faster spurge is controlled, the quicker land can be returned to more profitable uses.

Table 10. Site characteristics

County	SiteName	Vegetation	Shade	Duff (cm)	Drainage	Topography	Soil Texture
Beltrami	Odegaard	open field/prairie	none	0.25	well drained	level	sandy loam
Big Stone	Big Stone State Park 1	shrub-prairie mix	slight	0.00	well drained	level	silt loam
Big Stone	Big Stone State Park 2	shrub-prairie mix	slight	1.00	well drained	level	silt loam
Brown	Flandrau A	open field/prairie	none	3.00	moderately drained	level	sandy loam
Brown	Flandrau B	open field/prairie	none	4.00	well drained	hillside	clay loam
Brown	Flandrau C	open field/prairie	partial	1.00	well drained	hillside	loam
Brown	Flandrau D,G,H	open field/prairie	partial	1.00	well drained	level	sandy loam
Brown	Flandrau E	open field/prairie	none	3.00	moderately drained	level	sandy loam
Brown	Flandrau F	open field/prairie	partial	1.00	well drained	hillside	loam
Carlton	Douglas	open field/prairie	none	3.00	well drained	level	sandy loam
Carlton	Hidden Valley	open field/prairie	none	1.50	well drained	hillside	sandy loam
Cottonwood	#4	open field/prairie	slight	1.00	well drained	level	loam
Cottonwood	#7	open field/prairie	slight	1.00	well drained	hillside	silt loam
Crow Wing	Crow Wing Airport 1	open field/prairie	none	0.25	well drained	level	loamy sand
Crow Wing	Crow Wing Airport 2	shrub-prairie mix	none	0.00	well drained	level	loamy sand
Douglas	Pipo	open field/prairie	none	2.00	well drained	hillside	loam
Douglas	Zunker	open field/prairie	none	2.50	well drained	hillside	loam
Hennepin	Bryant Lake Park A	open field/prairie	slight	0.50	well drained	hilltop	loamy sand
Hennepin	Bryant Lake Park B	open field/prairie	none	1.00	well drained	hillside	sandy loam
Itasca	99-2	woodland/meadow	partial	0.50	well drained	hillside	loamy sand
Itasca	Northome A	open field/prairie	none	1.00	well drained	hillside	silty clay loam
Itasca	Northome B	open field/prairie	none	1.00	well drained	hilltop	silt loam
Kanabec	Ethel Hall Farm	shrub-prairie mix	slight	1.00	well drained	hillside	sandy loam
Kandiyohi	Bruce Peterson	open field/prairie	none	1.00	well drained	hillside	loam
Kandiyohi	County Landfill	open field/prairie	none	1.00	moderately drained	valley or swale	sandy loam
Kittson	Lake Bronson State Park	open field/prairie	slight	2.00	well drained	level	sandy loam
Koochiching	Dummick Lakes	woodland/meadow	slight	2.00	moderately drained	level	sandy loam
Lyon	Sioux Prairie WMA	wet prairie	slight	4.00	moderately drained	level	loam
Mille Lacs	Greenbush Pit	shrub-prairie mix	slight	2.00	well drained	level	sandy loam
Mille Lacs	Thielen	woodland/meadow	slight	1.00	well drained	level	sandy loam
Murray	Lake Shetek State Park	open field/prairie	none	2.00	well drained	level	clay loam
Nicollet	Hintz 95, 96	shrub-prairie mix	slight	4.00	moderately drained	valley or swale	sandy loam

County	SiteName	Habitat Type	Shade	Duff (cm)	Drainage	Topography	Soil Texture
Nicollet	Hintz 99-4	open field/prairie	none	3.00	well drained	hillside	sandy loam
Nicollet	Hintz A1	open field/prairie	none	2.00	well drained	hillside	sandy loam
Norman	Pinske	shrub-prairie mix	slight	0.00	moderately drained	hillside	sandy loam
Norman	Radneicki	woodland/meadow	slight	1.00	moderately drained	hillside	loamy sand
Pope	Glenwood	open field/prairie	none	1.00	well drained	hillside	loam
Ramsey	TCAAP 1	open field/prairie	none	0.00	well drained	hillside	loamy sand
Ramsey	TCAAP 22	open field/prairie	none	1.00	well drained	level	sandy loam
Rock	Tofteland 1	open field/prairie	none	1.00	moderately drained	level	silty clay loam
Rock	Tofteland 2	open field/prairie	none	1.00	moderately drained	level	silty clay loam
Stevens	Weiler WPA 1	wet prairie	none	0.50	poorly drained	level	loam
Stevens	Weiler WPA 2	wet prairie	none	0.25	poorly drained	level	loam
Stevens	Weiler WPA 3,4	wet prairie	none	0.50	poorly drained	level	clay loam
Swift	Appleton	open field/prairie	slight	1.00	well drained	level	loam
Swift	Jonathon Fahl	open field/prairie	none	0.50	well drained	hillside	silt loam
Traverse	Eggers	open field/prairie	none	2.00	well drained	hilltop	silt loam
Traverse	Fibranz	open field/prairie	none	3.00	moderately drained	hillside	silt loam
Wadena	Brockpahler	open field/prairie	partial	1.00	moderately drained	level	sandy loam
Wadena	Grondahl, Grassy Hill	woodland/meadow	moderate	2.00	well drained	hilltop	loamy sand
Wadena	Grondahl, Turkey Pen	open field/prairie	none	0.50	well drained	hilltop	loamy sand
Wadena	Sebeka Industrial Park	open field/prairie	none	3.00	well drained	level	sandy loam
Washington	William O'Brien State Park	open field/prairie	none	0.75	moderately drained	level	sandy clay loam
Wilkin	Jacklitch	open field/prairie	none	2.00	moderately drained	level	silty clay loam
Wilkin	Miller	shrub-prairie mix	moderate	1.00	moderately drained	level	silty clay loam
Winona	Prairie Island 1	wet prairie	partial	0.00	poorly drained	level	sandy loam
Winona	Prairie Island 2	shrub-prairie mix	none	2.00	poorly drained	level	sandy loam
Winona	Prairie Island 3	wet prairie	none	1.00	moderately drained	level	loamy sand
Winona	Prairie Island 4	wet prairie	none	0.50	poorly drained	level	loam

Table 11. *Aphthona* spp. release history and species recovery for each site. Date and quantity columns refer to release information.

County	Site	Release Date	Quantity	Species Released*	Species Recovered
Beltrami	Odegaard	07/96	2,500	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
		07/97	20,000	*A. lacertosa, A. czwalinae*	
		07/97	2,000	*A. nigriscutis, A. flava*	
		06/98	57,000	*A. lacertosa, A. czwalinae*	
Big Stone	Big Stone State Park 1	06/98	5,000	*A. lacertosa*	*A. lacertosa*
Big Stone	Big Stone State Park 2	06/98	3,000	*A. lacertosa*	*A. lacertosa*
Brown	Flandrau A	07/92	500	*A. flava*	*A. lacertosa, A. flava*
		08/93	500	*A. flava*	
		07/96	1,500	*A. flava*	
Brown	Flandrau B	07/92	500	*A. nigriscutis*	*A. lacertosa*
Brown	Flandrau C	08/96	2,000	*A. nigriscutis*	*A. lacertosa*
Brown	Flandrau D,G,H	07/96	700	*A. lacertosa, A. czwalinae*	*A. lacertosa*
		07/96	2,000	*A. flava*	
Brown	Flandrau E	07/96	3,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Brown	Flandrau F	07/96	4,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Carlton	Douglas	07/99	40,000	*A. lacertosa, A. nigriscutis*	*A. lacertosa*
Carlton	Hidden Valley	06/99	35,000	*A. lacertosa*	*A. lacertosa*
Cottonwood	#4	06/99	6,500	*A. lacertosa*	*A. lacertosa, A. nigriscutis*
Cottonwood	#7	06/99	11,000	*A. lacertosa*	*A. lacertosa, A. nigriscutis*
Crow Wing	Crow Wing Airport 1	06/98	8,000	*A. lacertosa*	*A. lacertosa*
		06/99	70,000	*A. lacertosa*	
Crow Wing	Crow Wing Airport 2	06/99	70,000	*A. lacertosa*	*A. lacertosa*
Douglas	Pipo	06/98	4,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
		07/98	4,000	*A. nigriscutis, A. flava*	
		07/99	50,000	*A. lacertosa*	
Douglas	Zunker	06/98	1,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
		07/99	40,000	*A. lacertosa, A. czwalinae*	
Hennepin	Bryant Lake Park A	07/97	10,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
Hennepin	Bryant Lake Park B	07/97	15,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Itasca	99-2	07/99	5,000	*A. lacertosa*	*A. lacertosa*

County	Site	Date	Quantity	Species Released*	Species Recovered
Itasca	Northome A	06/96	1,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
		07/97	10,000	*A. lacertosa, A. czwalinae*	
Itasca	Northome B	07/97	20,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Kanabec	Ethel Hall Farm	06/99	50,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Kandiyohi	Bruce Peterson	06/98	7,500	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Kandiyohi	County Landfill	06/98	2,500	*A. lacertosa*	*A. lacertosa*
Kittson	Lake Bronson State Park	07/90	1,000	*A. nigriscutis*	*A. lacertosa*
		07/92	300	*A. flava*	
		06/98	12,000	*A. lacertosa, A. czwalinae*	
Koochiching	Dummick Lakes	07/99	160,000	*A. lacertosa*	*A. lacertosa*
Lyon	Sioux Prairie WMA	06/98	2,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
		07/98	2,250	*A. nigriscutis*	
Mille Lacs	Greenbush Pit	06/99	6,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Mille Lacs	Thielen	06/99	3,000	*A. lacertosa*	*A. lacertosa*
Murray	Lake Shetek State Park	06/98	3,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Nicollet	Hintz 95, 96	06/95	1,000	*A. nigriscutis*	*A. lacertosa, A. nigriscutis*
		07/96	300	*A. lacertosa, A. czwalinae*	
Nicollet	Hintz 99-4	06/99	4,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
Nicollet	Hintz A1	07/94	1,000	*A. nigriscutis*	*A. lacertosa, A. nigriscutis*
Norman	Pinske	06/98	2,000	*A. lacertosa, A. czwalinae*	*A. nigriscutis*
Norman	Radneicki	07/97	4,000	*A. lacertosa*	*A. lacertosa*
		06/98	2,000	*A. lacertosa*	
Pope	Glenwood	07/97	5,500	*A. lacertosa*	*A. lacertosa, A. nigriscutis*
		07/97	6,500	*A. nigriscutis*	
Ramsey	TCAAP 1	06/99	9,000	*A. lacertosa, A. nigriscutis*	*A. lacertosa, A. nigriscutis*
Ramsey	TCAAP 22	06/99	5,000	*A. lacertosa, A. nigriscutis*	*A. lacertosa, A. nigriscutis*
Rock	Tofteland 1	06/98	1,000	*A. nigriscutis*	*A. lacertosa*
Rock	Tofteland 2	06/98	3,000	*A. lacertosa*	*A. lacertosa*
Stevens	Weiler WPA 1	07/99	20,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Stevens	Weiler WPA 2	06/00	20,000	*A. lacertosa*	*A. lacertosa*

County	Site	Date	Quantity	Species Released*	Species Recovered
Stevens	Weiler WPA 3,4	06/00	30,000	*A. lacertosa*	*A. lacertosa*
Swift	Appleton	06/98	7,000	*A. lacertosa*	*A. lacertosa, A. nigriscutis*
Swift	Jonathon Fahl	07/97	5,000	*A. lacertosa, A. czwalinae*	*A. lacertosa, A. nigriscutis*
		06/98	3,000	*A. lacertosa*	
		06/00	53,000	*A. lacertosa*	
Traverse	Eggers	06/98	1,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
Traverse	Fibranz	07/97	3,000	*A. lacertosa, A. czwalinae*	*A. lacertosa*
		06/98	17,000	*A. lacertosa, A. czwalinae*	
		06/99	20,000	*A. lacertosa*	
		07/99	40,000	*A. lacertosa*	
Wadena	Brockpahler	07/94	550	*A. nigriscutis*	*A. lacertosa, A. nigriscutis*
		06/96	1,000	*A. nigriscutis*	
		07/97	500	*A. lacertosa, A. czwalinae*	
		07/98	3,000	*A. nigriscutis*	
		06/99	40,000	*A. lacertosa*	
		07/99	30,000	*A. lacertosa*	
Wadena	Grondahl, Grassy Hill	07/99	1,300	*A. lacertosa*	*A. lacertosa*
Wadena	Grondahl, Turkey Pen	06/96	2,000	*A. nigriscutis*	*A. nigriscutis*
		07/97	1,000	*A. lacertosa, A. czwalinae*	
		07/98	4,000	*A. nigriscutis*	
		07/99	20,000	*A. lacertosa*	
Wadena	Sebeka Industrial Park	06/96	2,000	*A. nigriscutis*	*A. lacertosa, A. nigriscutis*
		07/99	71,000	*A. lacertosa*	
Washington	William O'Brien State Park	06/98	4,000	*A. lacertosa*	*A. lacertosa*
		06/00	20,000	*A. lacertosa*	
Wilkin	Jacklitch	07/95	1,000	*A. nigriscutis*	*A. lacertosa, A. flava*
		06/96	2,000	*A. lacertosa, A. czwalinae*	
		06/98	2,000	*A. lacertosa, A. czwalinae*	
		06/99	60,000	*A. lacertosa*	
		07/99	99,000	*A. lacertosa, A. nigriscutis*	

County	Site	Date	Quantity	Species Released*	Species Recovered
Wilkin	Miller	06/97	5,000	*A. lacertosa*	*A. lacertosa, A. flava*
		07/97	2,500	*A. lacertosa, A. czwalinae*	
		06/98	2,000	*A. lacertosa, A. czwalinae*	
		06/98	7,000	*A. lacertosa, A. czwalinae*	
		06/99	40,000	*A. lacertosa*	
		07/99	41,000	*A. lacertosa*	
Winona	Prairie Island 1	07/96	2,000	*A. cyparissiae*	*A. lacertosa, A. flava*
		07/97	3,000	*A. lacertosa, A. nigriscutis*	
Winona	Prairie Island 2	07/96	2,000	*A. cyparissiae*	No *Aphthona* recovered
		07/97	2,000	*A. nigriscutis*	
		06/98	2,000	*A. lacertosa*	
Winona	Prairie Island 3	07/96	2,000	*A. cyparissiae*	No *Aphthona* recovered
		07/97	2,000	*A. nigriscutis*	
Winona	Prairie Island 4	07/97	2,500	*A. nigriscutis*	No *Aphthona* recovered

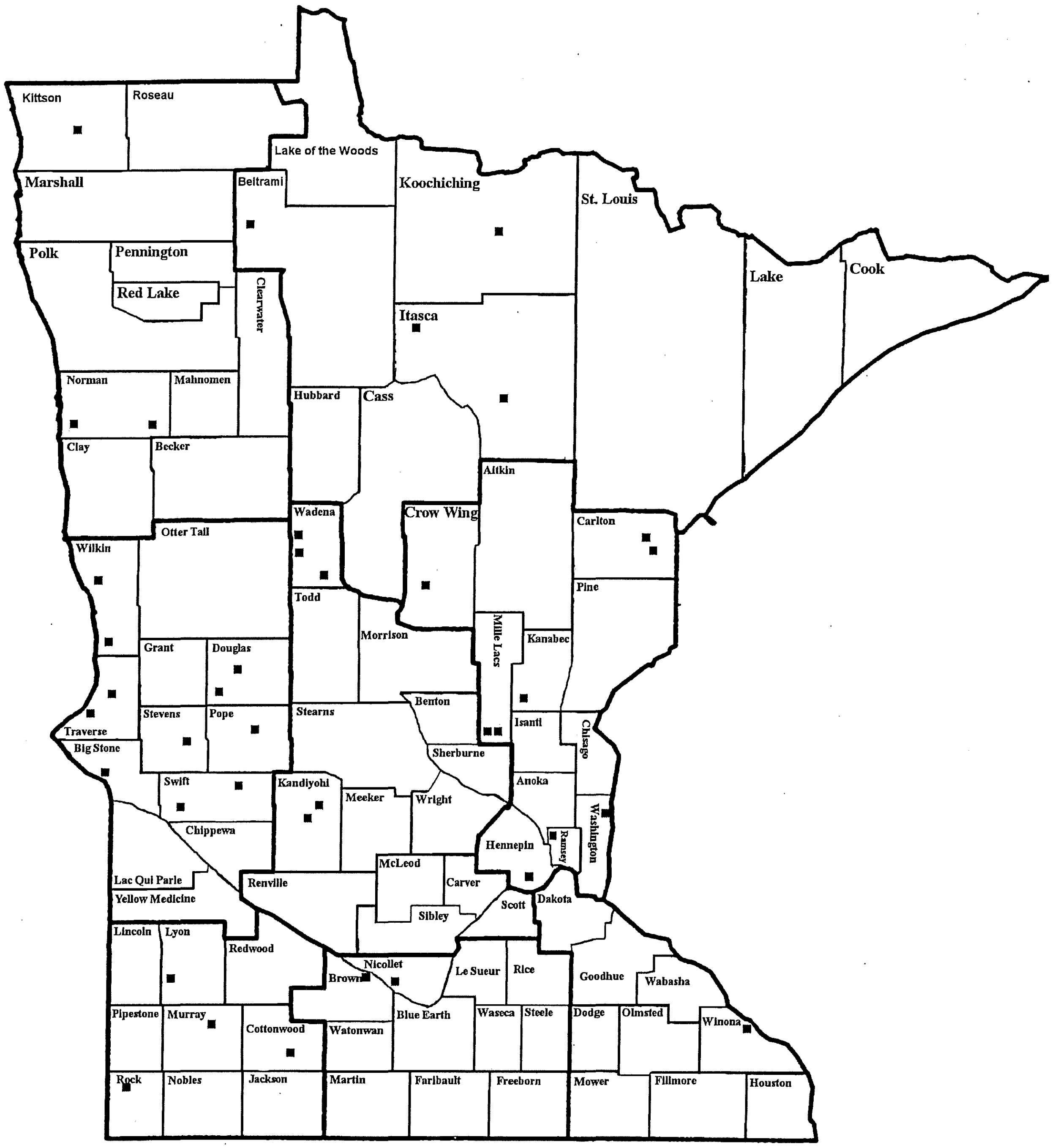

Figure 7. Site locations are marked with a square. A single square may denote multiple sites at a given location.

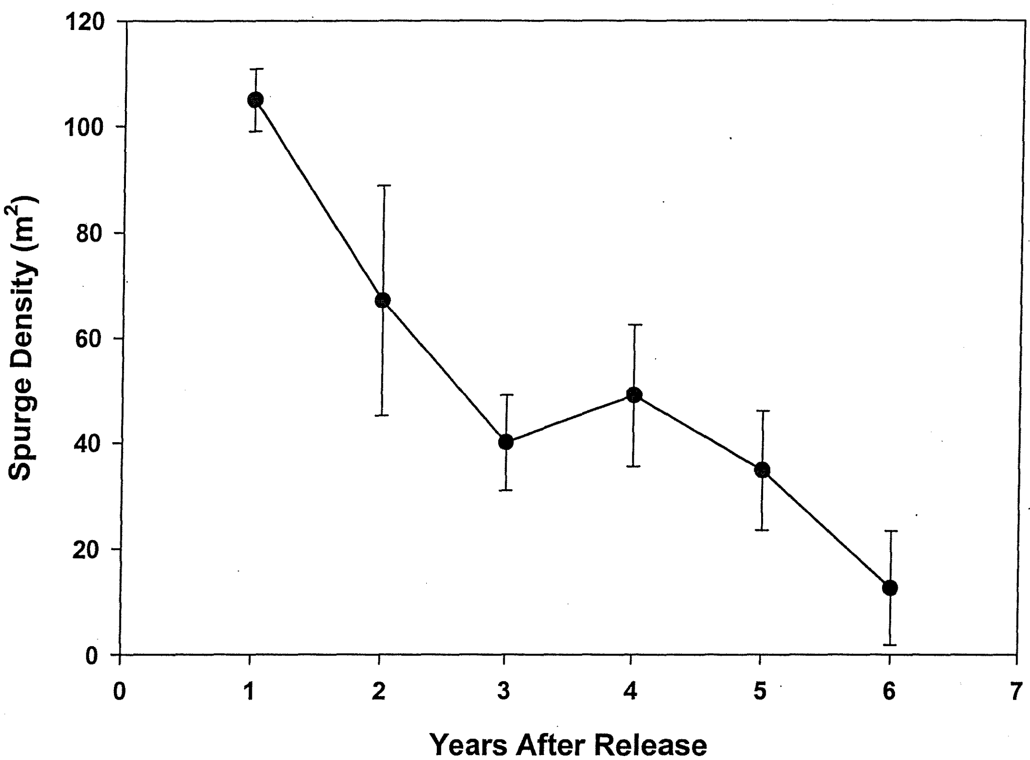

Figure 8. Mean spurge density for sites by number of years after final *Aphthona* release.

Objective 2. Test for interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis*

Introduction

Anecdotal evidence suggests that *Aphthona nigriscutis* populations have remained at low levels or became locally extinct in the presence of *A. lacertosa*. The rationale is that *A. lacertosa* populations tend to build quickly potentially affecting establishment of other introduced *Aphthona* species, where mixed releases occur. In many of the release sites, it is unknown how *A. nigriscutis* populations would respond in the absence of *A. lacertosa*.

Researchers have tried to link abiotic factors (climate, soil type, soil moisture, slope, aspect etc.) and biotic factors (leafy spurge density, leafy spurge bio-type, and vegetation community structure) to the establishment and growth of *Aphthona* populations (Hansen et al. 1997, Jordan 1999, Maw 1981 and Nowierski et al. 2002). However, researchers have not considered interspecific competition as a factor that may influence flea beetle populations. It may be that interspecific competition is too weak or sporadic to be an important mechanism that influences community structure of phytophagous insects (Strong et al. 1984) in our system. Strong et al (1984) believed that resources were rarely limiting for phytophagous insects because of suppression by predators and parasitoids. Denno et al. (1995) provided evidence to the contrary. They reviewed 193 pair-wise phytophagous species interactions and found that 76% were considered interspecific competition. Denno et al. (1995) claimed that phytophagous insects were more likely to compete if they were closely related, introduced, sessile, aggregative, fed on discrete resources, and fed on forbs or grasses. These factors provide support to the idea that interspecific competition may occur between *Aphthona lacertosa* and *A. nigriscutis*. These two flea beetles are closely related and occupy the same feeding niche on leafy spurge (Maw 1981). Both species are introduced, leaving their natural enemies behind, thus reducing the importance of vertical trophic interactions (Gassmann et al. 1996, Hansen et al. 1997). *Aphthona lacertosa* and *A. nigriscutis* are monophagous or at most narrowly oligophagous species and their larvae attack a discrete resource (the roots of leafy spurge).

There are several examples of interspecific competition between two or more insects introduced as agents for perennial weed control. The systems include *Urophora afftnis and U. quadrifasciata* (Diptera: Tephritidae) on diffuse knapweed, *Centaurea diffuse* (Berube 1980); *Metzneria paucipuntella* (Lepidoptera: Gelechiidae), *U. affinis and U. quadrifasciata* on spotted knapweed, *Centaurea maculosa* (Story et al. 1991); *Tyriajacobaeae* (Lepidoptera: Arctiidae) and *Pegohylemia seneciella* (Diptera: Anthomyiidae) on tansy ragwort *Senecio jacobaea* (Crawley and Pattrasudhi 1988); and *Rhinocyllus conicus* (Coleoptera: Curculionidae) and *U. solstitialis* (Diptera: Tephritidae) on nodding thistle, *Carduus nutans* (Woodburn 1996). All four weed species listed above are found in rangeland habitats similar to leafy spurge.

To determine if competition is occurring between the two *Aphthona* species, we tested the hypothesis that interspecific competition is occurring whereby *A. nigriscutis* replacement rates are significantly lower in the co-occurring populations compared to *A. nigriscutis* alone and *A. nigriscutis* replacement rates are lower than *A. lacertosa* in the co-occurring populations.

Materials and Methods

A substitutive experimental design was used to test our hypothesis. Since *A. lacertosa* and, *A. nigriscutis* are univoltine, the experiments were conducted over two field seasons to capture replacement rates of each species within treatments. Twelve cages (2m by 2m in size) were established at one field site. We chose a site with sandy soil to provide *A. nigriscutis* a suitable habitat type based on the literature (Rees et al. 1996, Nowierski 2002). Cages were placed over patches of leafy spurge with similar densities as determined by leafy spurge stem counts in each plot prior to treatment. All plots were placed within the same spurge patch. Three treatments including 100 *A. lacertosa* only, 100 *A. nigriscutis* only and a combination of 50 *A. lacertosa and* 50 *A. nigriscutis* were applied to four cages each in a randomized design. Both species of *Aphthona* were collected from the field and sorted 24 hours prior to treatments. One year post treatment, each cage was swept (7 sweeps per cage with standard 38 cm. diameter sweep net) weekly during June and July to capture peak abundance. The number of each *Aphthona* species were counted and returned to the cage. Leafy spurge stems were counted pretreatment and one year post treatment in each plot.

Replacement rates were calculated for each treatment. Replacement rate is defined as the number of offspring each *Aphthona* spp. produced divided by number of individuals introduced into each treatment. Replacement rates of *A. lacertosa* and *A. nigriscutis* were treated independently in the 50/50 treatment. The replacement rates were arcsine transformed prior to analysis. An ANOVA was applied to the data set using SAS to test significant differences between treatments.

Results

One year post treatment, there were no significant differences between treatment replacement rates. (Figure 9). The mean replacement rate for all *Aphthona* species and treatments was 0.56. Replacement rates across all treatments ranged from 0.07 to 0.92.

Leafy spurge stem densities significantly decreased one-year post treatment for all treatments. (Figure 10). Prior to treatment the mean leafy spurge stem density per plot was 239.6. Leafy spurge densities per plot ranged from 178 to 301 stems. Leafy spurge stem densities, however, significantly decreased one-year post treatment (Figure 10). The mean stem density per plot across all treatments was reduced to 86.4 stems, and stem density ranged from 6 to 123.

Discussion

The lack of treatment effects on replacement rates suggests that interspecific and intraspecific competition are occurring at the same rate. We chose a habitat type that would favor *A. nigriscutis*, as to not give an advantage to the more dominant species, *A. lacertosa*. Frequency dependence can be used to assess interspecific competition (deWit 1960, 1961; Ayala 1971). Our data suggest that there were higher frequencies of *A. nigriscutis* than *A. lacertosa* (Figure 11). Although Figure 11 does not imply significance, it demonstrates that the *Aphthona* populations did not behave as we might have expected. The highest replacement rates came from *A. nigriscutis* only treatments, which is contrary to what we expected. *Aphthona lacertosa* was expected to be the dominant species if not competitively, at least in numbers of individuals.

Within this habitat, *A. nigriscutis* seem to be as productive and reduce leafy spurge densities at similar rates as *A. lacertosa*. Interspecific competition may not have occurred due to lack of limited resources. Strong et al (1984) concluded that for competition to occur, resources must be limited. In our study, one year after treatment, an average of 86 leafy spurge stems per plot remained. If interspecific competition is to take place, it might not be evident until two years post treatment when leafy spurge densities may be limiting. It will be important to assess the plots a second year to document any changes in species abundance and to determine if replacement rates of *A. nigriscutis* remains high in the presence of a limited resource.

If interspecific competition is not playing a role in *A. nigriscutis* establishment, we could speculate that habitat types may be the major influence on their establishment and control success. In Minnesota, the majority of the site types tend to be mesic to moderately dry sites with clay-loam soils. These types of sites tend to favor *A. lacertosa* (Rees et al. 1996 and Nowierski 2002). This study was conducted on only one site type. It would be instructive to repeat this experiment in habitats that are more favorable to *A. lacertosa* and to apply higher rates of insects or to have release rates based on stem densities rather than a static number of insects per unit area to ensure a limited leafy spurge resource in the first year. Revelations of interspecific competition could change management practices by releasing only one species per site if warranted.

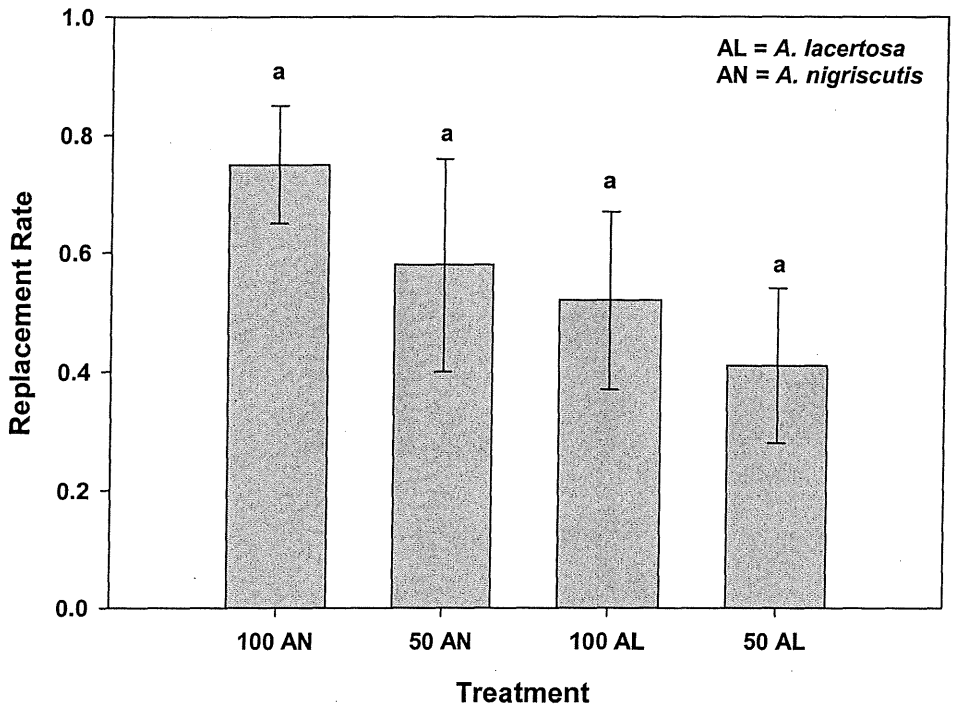

Figure 9. Replacement rates of *Aphthona* one year post treatment. There were no significant differences by treatment as indicated by the lower case letters.

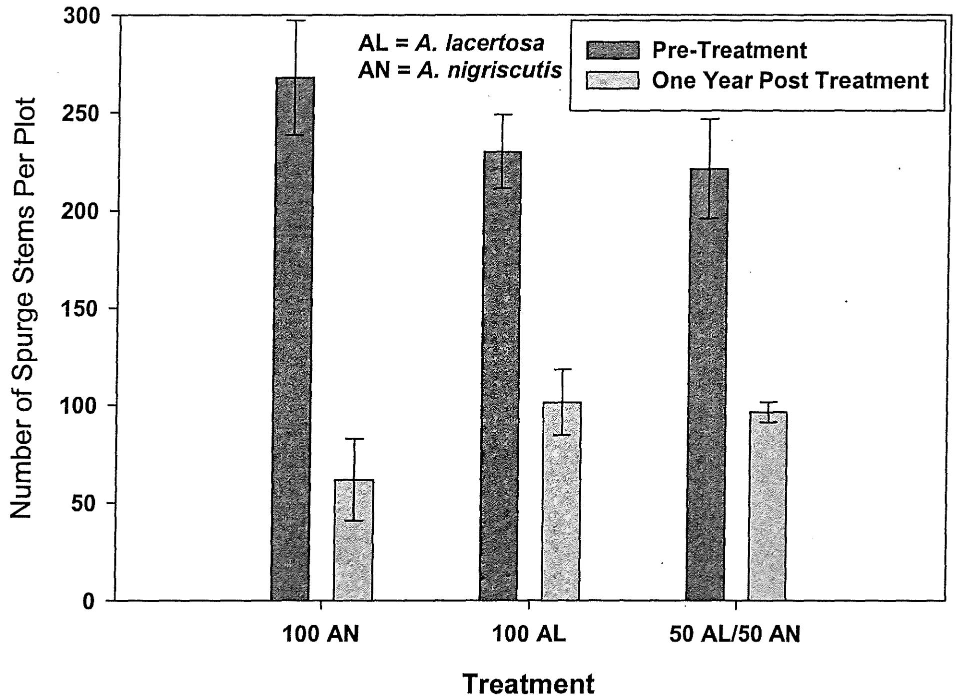

Figure 10. Mean number of leafy spurge stems pretreatment and one year post-treatment

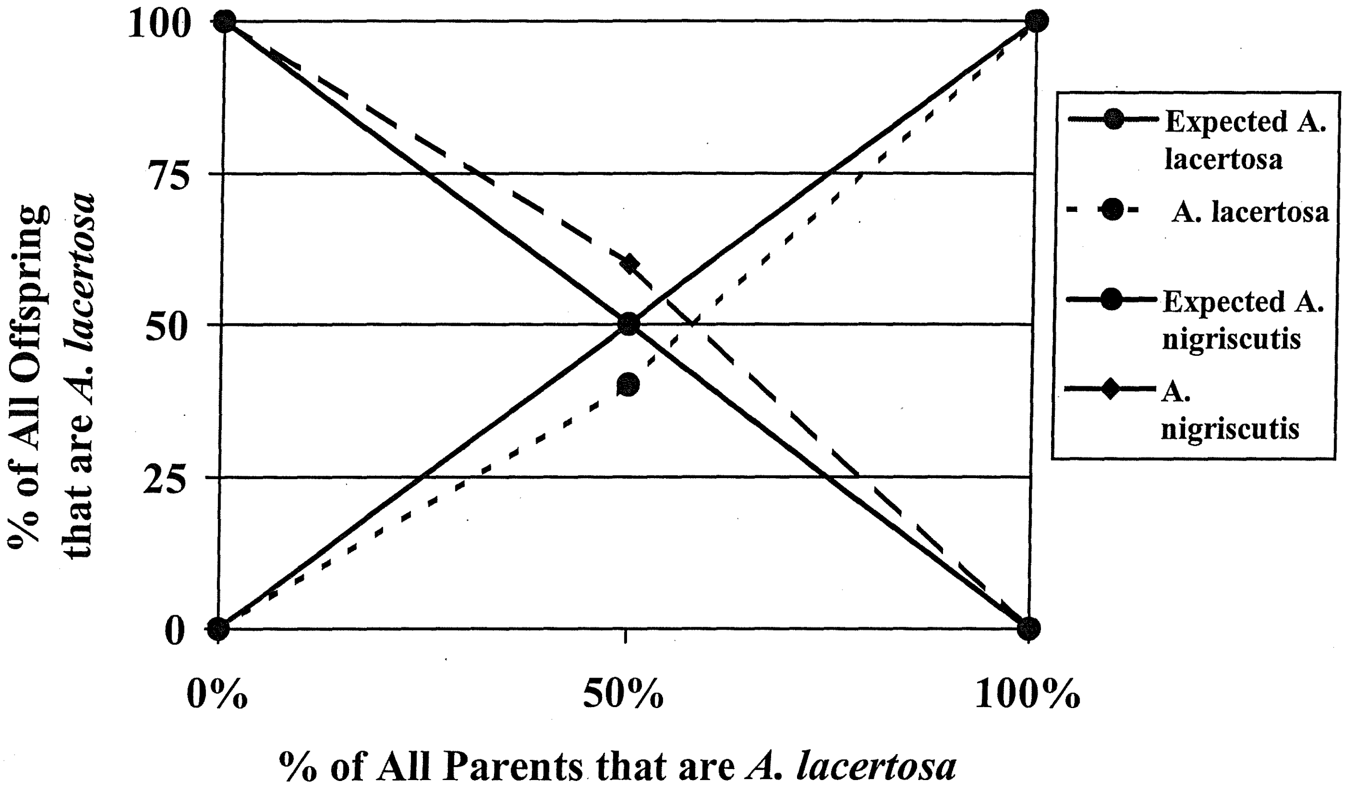

Figure 11. Frequency dependence of *A. lacertosa* and *A. nigriscutis* one year after treatment. Solid lines are what is expected to occur if interspecific competition is not occurring. Dashed lines are actual data from *Aphthona* replacement experiment.

Objective 3. Test effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis*

Introduction

Initial *Aphthona* species releases in Minnesota tended to be small quantities (less than 1,000 beetles) due to large numbers of potential release sites and a lack of available flea beetles. *Aphthona* spp. were shipped from locations as far away as Montana. Many of these initial releases did not establish successfully. It is unclear whether these releases failed because of the small release size, condition of the beetles after shipping, or other biotic and abiotic factors associated with the release sites.

Aphthona lacertosa is the predominant species in Minnesota followed by *A. nigriscutis*. *Aphthona nigriscutis* often appears in conjunction with *A. lacertosa* in the field. *Aphthona* spp. are gregarious (Lym et al. 1999) and require sufficient numbers to reproduce and colonize a new site. However, minimum release quantity for successful establishment has not been determined. It is also not clear whether the minimum release quantity differs by *Aphthona* species. Lym et al. (1999) suggests that release quantities of 1,000 flea beetles may be needed to ensure establishment. Although larger numbers of flea beetles (>2000 beetles) are currently being released on individual sites for leafy spurge management, it is important to understand how release quantity affects establishment and control success. The purpose of this study is to test the effect of release quantity on establishment and control by *A. lacertosa* and *A. nigriscutis*.

Materials and Methods

Thirty-six circular plots with 20 meter diameters were established in June 2000 at a field site in Otter Tail County (Fig. 12). Six treatments of two *Aphthona* species were released on 06/21/00. Treatments were randomly assigned to plots and included: Control (no beetles released), 250 *A. lacertosa*, 250 *A. nigriscutis*, 1,000 *A. lacertosa*, 1,000 *A. nigriscutis* and 500 *A. lacertosa*/500 *A. nigriscutis*. Each treatment was replicated six times. Three soil cores across the experimental area were taken. Soil from cores were homogenized and processed by the University of Minnesota Soil Testing Laboratory for a textural analysis.

Aphthona spp. were sampled weekly for eight weeks during the 2001 field season using a standard 15 inch diameter sweep net. A sample consisted of 10 pendulum sweeps and five samples were taken in each plot. The number of *Aphthona* spp. collected was recorded and beetles were immediately returned to the plot.

Vegetation data were taken once per season during late June each of the two field seasons. The 2000 data were taken prior to insect release. Five 0.25 m^2 samples per site were selected randomly using a square. For each sample we counted the number of flowering spurge stems, number of non-flowering stems, height of the five tallest stems, along with an estimate of percent cover due to spurge and percent cover of all other vegetation. Percent cover was categorized as 0, 1-5, 6-25, 26-50, 51-75, and 76-100%. Vegetation other than spurge was further categorized as annual grass, annual or biennial forb, perennial forb, perennial grass, and woody perennial.

The number of *Aphthona* spp. at peak emergence was compared between treatments by species using ANOVA by Statistica (StatSoft, Inc.). Number of spurge plants, spurge height, and spurge percent cover were compared between 2000 and 2001 within each treatment also by ANOVA.

Results

Within treatments, *A. lacertosa* established at all plots and *A. nigriscutis* was recovered from all but one plot. At peak emergence, the total number of *A. lacertosa* per plot ranged from 9 to 188 compared to *A. nigriscutis* ranging from 0 to 69 per 50 sweeps. Neither species affected the establishment of the other in the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatment.

The 1,000 *A. lacertosa* treatment produced the highest insect density that was significantly higher than all treatment except the combined 500 *A. lacertosa*/500 *A. nigriscutis* release rate ($P = 0.05$, Fig. 14). In contrast, there were no differences among the release rates of *A. nigriscutis*.

All plots initially contained spurge with a mean number of total (flowering and non-flowering stems) stems ranging from 42 to 169 stems per m^2 (Fig. 13). Density of flowering, non-flowering, or total number of spurge stems in general was not significantly different for most treatments ($P = 0.05$, Table 13). In 2001, mean number of spurge stems across all treatments ranged from 26 to 110 and 1 to 89 stems per m^2 for flowering and non-flowering spurge respectively. Significant reductions in the total number of spurge stems were observed in the 1,000 *A. nigriscutis*, 250 *A. lacertosa*, and the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatments. Spurge height ranged from 15 to 97 cm with a mean of 65.9 cm which was not different from stem height prior to release and one treatment, 250 *A. lacertosa,* showed a significant increase in stem height. Mean percent spurge cover for all plots ranged from 6-25% to 76-100%. The only significant change was an increase in spurge cover with the 1,000 *A. nigriscutis* treatment. The mean number of plant species per plot other than spurge was 1.6 species. Perennial grasses were the predominant vegetation type found in the plots followed by perennial forbs, annual or biennial forbs, and woody perennials respectively (Table 12).

Table 12. Categories of plant species other than leafy spurge found during vegetation surveys.

Category	**2000**	**2001**
annual or biennial forb	8.65%	6.85%
perennial grass	62.63%	83.06%
perennial forb	25.95%	9.68%
woody perennial	2.77%	0.40%

Discussion

In this study, we found that all three release rates resulted in successful establishment of both species, one year following release. The lack of treatment differences one year following release may be caused by too high initial release rates. Small insect populations may be subject to stochastic effects that may cause populations to go locally extinct. Measuring quantities of offspring two and three years after release may give a better indication of increasing or decreasing *Aphthona* populations rather than one year after release as measured in this study.

The site had a soil texture (sandy loam comprised of 68.4% sand, 20.0% silt and 11.6% clay) and general habitat conducive to the establishment of both *A. lacertosa* and *A. nigriscutis*. *Aphthona nigriscutis* prefers sandy, well-drained sites while *A. lacertosa* tolerates more mesic conditions (Gassman et al. 1996). *Aphthona nigriscutis* have difficulty establishing where leafy spurge density is greater than 320 stems/m^2 (Lym 1998) which is much denser than the maximum plot mean density of 183 stems/m^2 measured at this site. Yet less than five *A. nigriscutis* per plot were recovered from half of the exclusively *A. nigriscutis* treatment plots. *Aphthona nigriscutis* adults collected and released in 2000 may have oviposited prior to collection and redistribution, thereby limiting the number of eggs laid at the experimental sites. Alternatively, the relative success of the 1,000 *A. lacertosa* treatment supports that at higher release quantities, *A. lacertosa* may be more effective than *A. nigriscutis* at establishment at this site. The 250 *A. lacertosa* treatment proportionally produced a greater number of offspring per adult beetle released than the 1,000 *A. lacertosa* treatment. Data collected in 2002 will be used to corroborate the effect of founder population size on successful establishment and elucidate treatment differences. Establishment of either species was not adversely affected when a mixed population of *A. lacertosa* and *A. nigriscutis* were released in the same plots. With an abundant food supply we would not expect any interspecific competition to occur in these mixed releases. The higher *A. lacertosa* densities compared to *A. nigriscutis* found in these plots could be due to a higher reproductive rate of *A. lacertosa* or the quality of *A. lacertosa* collected in 2000 exceeded that of *A. nigriscutis*.

There were few significant differences in spurge density by treatment and these differences do not correlate with *Aphthona* densities. The 1,000 *A. nigriscutis* treatment showed decreases in both number of flowering and total number of spurge plants, but an increase in percent spurge cover which is likely due to slightly larger spurge plants. There was a background effect of an incorrect *Aphthona* species appearing in plots of exclusive treatments with the 250 *A. nigriscutis* treatment plots most affected. Therefore, for this treatment, any change in spurge density may not be attributed solely to *A. nigriscutis*. Visual observations of the plots revealed areas of spurge control often near the release point for *Aphthona* spp. treated plots. In summary, all treatments established at very low numbers and it is unknown whether these populations will increase and as a result, reduce spurge densities in the future. The continuation of this study will shed more light on this issue. The general practice of introducing four to five thousand *Aphthona* per site as recommended by the Minnesota Department of Agriculture seems to be a sound management practice to ensure establishment.

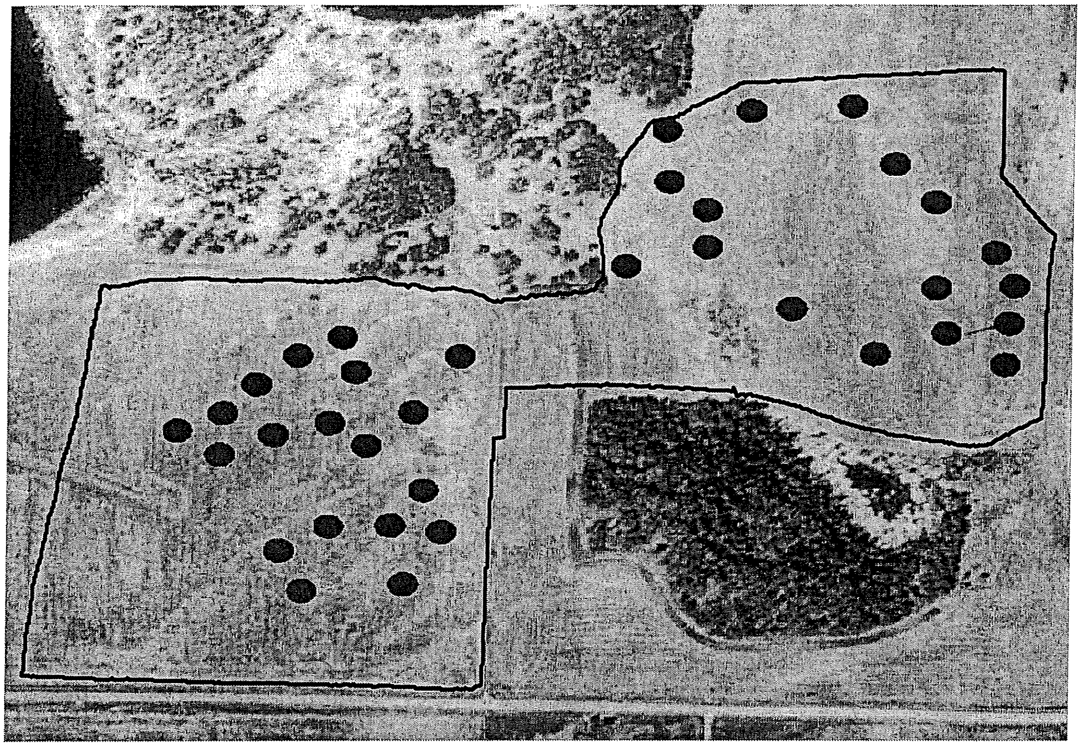

Figure 12. Aerial photo of research site with plots marked as solid dots.

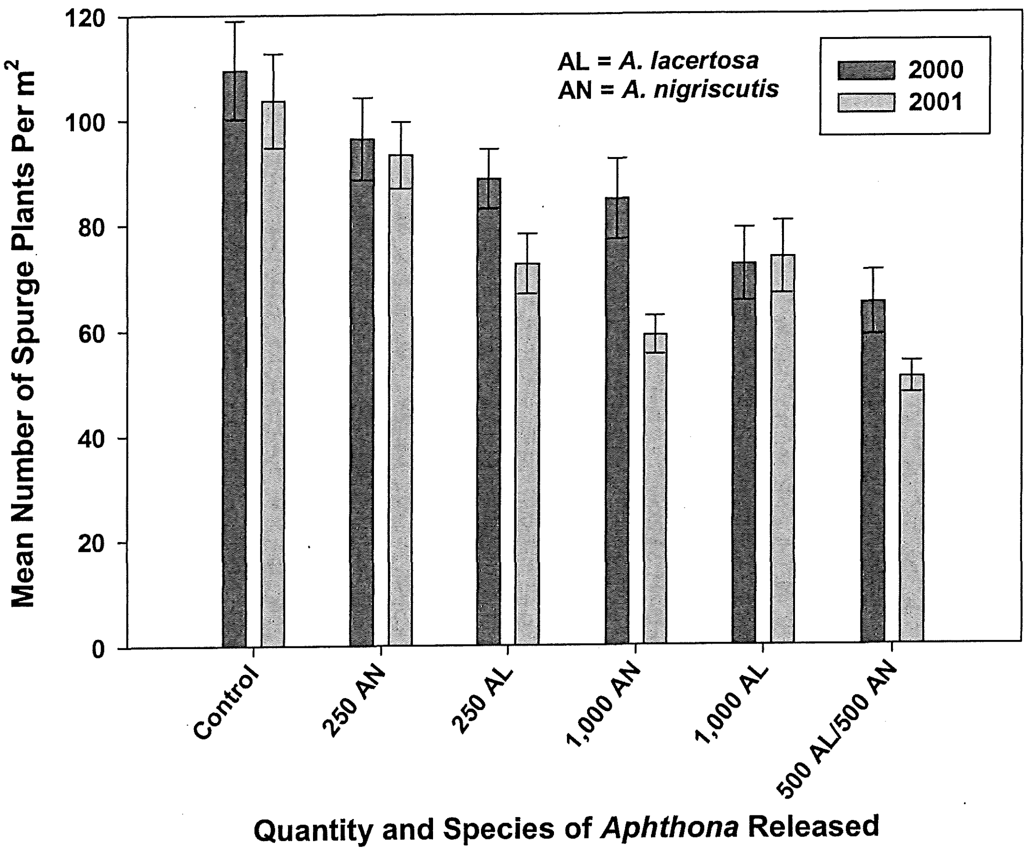

Figure 13. Mean and standard error for spurge density as determined by the number of both flowering and non-flowering spurge stems per m^2.

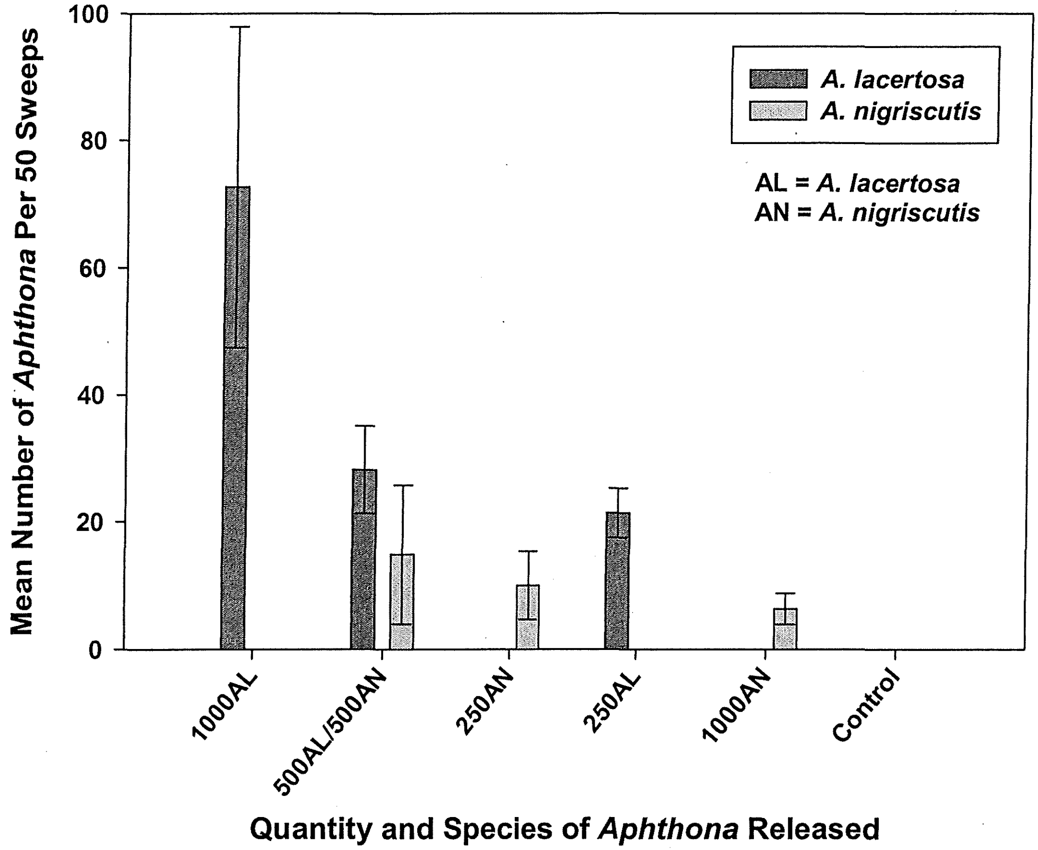

Figure 14. Mean and standard error for *Aphthona* recovered at peak emergence by species and treatment.

Table 13. Comparison of 2000 to 2001 means with standard errors. Significant differences (*P*=0.05) are marked in bold. Each comparison is made within a treatment, not between treatments. These data are based on m^2 samples.

Treatment	Measure	2000 Mean	2001 Mean	p
1000AL	Flowering	52.40±4.70	50.93±4.96	0.8307
1000AN	Flowering	59.87±5.22	41.20±3.50	**0.0043**
250AL	Flowering	65.60±4.93	52.80±5.25	0.0806
250AN	Flowering	66.80±5.64	61.20±4.93	0.4580
500AL/500AN	Flowering	46.53±5.74	37.60±3.55	0.1908
Control	Flowering	65.33±4.83	68.00±6.74	0.7489
1000AL	Non-flowering	19.87±5.67	22.67±4.64	0.7036
1000AN	Non-flowering	24.80±5.12	17.73±3.70	0.2679
250AL	Non-flowering	22.93±2.83	19.60±2.84	0.4093
250AN	Non-flowering	29.47±6.12	31.87±5.57	0.7730
500AL/500AN	Non-flowering	18.40±2.47	13.20±2.39	0.1355
Control	Non-flowering	44.13±7.43	35.60±5.57	0.3619
1000AL	Total Spurge	72.27±6.91	73.60±6.85	0.8915
1000AN	Total Spurge	84.67±7.65	58.93±3.61	**0.0035**
250AL	Total Spurge	88.53±5.67	72.40±5.64	**0.0484**
250AN	Total Spurge	96.27±7.90	93.07±6.36	0.7536
500AL/500AN	Total Spurge	64.93±6.11	50.80±3.01	**0.0425**
Control	Total Spurge	109.47±9.45	103.60±9.03	0.6553
1000AL	Height	63.41±1.56	63.29±2.05	0.9643
1000AN	Height	62.55±2.02	65.88±2.14	0.2631
250AL	Height	63.73±1.60	70.35±1.69	**0.0060**
250AN	Height	62.08±1.81	63.29±1.88	0.6430
500AL/500AN	Height	61.12±2.21	63.70±2.39	0.4319
Control	Height	65.66±1.29	69.14±2.18	0.1739

Objective 4. Develop a day-degree emergence model for *Aphthona* spp. adults

Introduction

Understanding phenological patterns of *Aphthona* flea beetles, in particular predicting peak abundance, provides researchers and land managers information on when to collect agents for redistribution, assess population establishment and carry out other management strategies as part of an integrated pest management program. To develop this phenology model, three steps were required: 1) determine the lower developmental threshold for each species, 2) estimate peak emergence in the field for each species using accumulated degree-days (ADD), and 3) develop maps of the state that spatially represent predicted occurrence of peak abundance. The first step in developing phenological models is to determine developmental rates and estimate lower developmental thresholds for *A. lacertosa* and *A. nigriscutis*. The functional lower developmental threshold for *A. nigriscutis* has been estimated using field collected data and modeled with CALFUN (Legg et al. 2002). Determining development rates and lower developmental thresholds for introduced *Aphthona* spp. in controlled laboratory experiments is difficult because the third instar larvae overwinter in the roots of the host plant and are univoltine. The first objective of this study is to assess the effect of selected constant temperatures on the development of *A. lacertosa* and *A. nigriscutis* from third instar to adult emergence. The second objective is to estimate peak emergence for each species using accumulated degree-days based on the historical method and the lower developmental threshold determined in objective one. The third objective is to incorporate the insect phenology models with GIS analysis to develop maps that spatially represent predicted peak abundance of *A. lacertosa* and *A. nigriscutis*.

Materials and Methods

Developmental Rates and Lower Developmnental Threshold Determination

For this study we determined developmental rates based on development time from third instar (overwintering) larvae to adult emergence. This stage of development was chosen due to interest in adult emergence patterns in the field. Soil cores containing leafy spurge roots with *Aphthona* spp. larvae were collected from three field sites using a golf course cup cutter (10 cm. diameter by 15-20 cm. depth) in November 2000 and 2001, prior to frost set in soil. Three sites were chosen to ensure capture of *A. lacertosa* and *A. nigriscutis*. Soil cores collected in 2000 were held in a cold chamber at 3° C for 60 days prior to treatment. Soil cores were placed in emergence traps made of one-half gallon paper container topped with an inverted plastic funnel and capped with a clear collection jar. Forty five soil cores (15 from each location) were randomly assigned to one of five constant temperature regimes in five growth chambers (Coviron) held at 15,18, 21, 22.5, and 26°C with 24 hour light. Two temperature data loggers were placed within two separate soil cores per chamber to verify chamber temperature. For each temperature regime, the date and quantity of each species of newly emerged adults was recorded daily for each collection site. Mean number of days to emergence and standard errors were calculated for each temperature and collection site. The experiment was repeated (using soil cores collected in November 2001) at 6, 9, 12, 15, 18, 21 and 24° C to further define development rates at lower temperatures and more accurately predict the lower developmental thresholds. Datasets for 2000 and 2001 were combined after no significant differences in slopes and intercepts of fitted regressions were found. This procedure was completed for *A. lacertosa* and *A. nigriscutis* separately. Lower developmental thresholds (T_0) were estimated by plotting rate of development (1/d, where d=time in days) versus Temperature (T, ° C) then applying a linear regression to the dataset. The regression was tested for non-constant variance and curvature to ensure linearity. T_0 was calculated by setting Y (1/d) equal to zero and solving for X (T, ° C) in the linear regression equation ($T_0 = -a/b$). The reciprocal of the slope (1/b) estimates accumulated degree-days required for development. Regression coefficients, estimates and standard errors were all calculated using Arc statistical regression software (Cook and Weisberg 1999).

Phenology Model

For this study, twenty-six sites were selected in three counties with 16 sites in Clay, 8 sites in Otter Tail, and 2 sites in Becker (Figure 1). The release history for each site is similar (Table 5) in that *Aphthona* spp. were introduced in all sites between 1997 and 1999 with a similar number of *Aphthona* released with one exception of a higher release number. *Aphthona* were sampled weekly for eight weeks during the 2000 and 2001 field seasons. Using a standard 38 cm. diameter sweep net and 10 sweeps per sample, a designated number between 6 and 10 samples depending on the size of the site were taken for each site. The beetles were placed in plastic bags then frozen until they could be separated by species and counted. The percent maximum capture for each species was calculated for each site and sampling date in 2000 and 2001. The data were pooled by sampling date, for each year and county, with Clay and Becker county sites combined due to close proximity.

Accumulated degree-days (historical method) were calculated using previously determined lower developmental thresholds (Table 17) and local weather station daily temperature data. One weather station was used for the Clay and Becker county sites and a second used for the Otter Tail county sites. For each species, percent maximum capture versus accumulated degree-days was plotted. A non-linear, third order polynomial was fitted to the regressions to approximate seasonal abundance similar to models suggested by Dennis et al. (1986). The accumulated degree-days to peak abundance were calculated by solving for the maximum value (percent maximum capture) on the fitted curve (Table 17). Regression coefficients, estimates and standard errors were all calculated using Arc statistical regression software (Cook and Weisberg 1999).

Displaying Models Spatially

To develop maps that spatially represent predicted occurrence of peak abundance for *A. lacertosa* and *A. nigriscutis*, adjusted normal (30 year average) temperature data from 172 weather stations was used (Fig. 20, NOAA 2001). Normal monthly temperature data was used to calculate average daily temperatures (Greg Spoden, pers. comm.). For each weather station, the normal daily temperatures were used to calculate accumulated degree-days using the historical method based on predetermined lower developmental thresholds for each *Aphthona* species (Table 15). The average julian date to peak abundance was determined for each weather station based on previously estimated ADD to peak abundance. Geographic Information System software (Surfer) incorporated the Julian date values and associated weather station coordinates to spatially display estimated peak abundance by calendar date. A Kriging interpolation was used in map development.

Results

Results

Developmental Rates and Lower Developmnental Threshold Determination
A total of 3,355 *A. lacertosa* individuals were collected from the growth chambers while only 277 *A. nigriscutis* individuals were collected. Developmental times decreased with increasing temperatures (Table 14). Average days to emergence ranged from 68.6 days at 15°C to 25.6 days at 26°C for *A. lacertosa* and 84.3 days at 15°C to 26.1 days at 26°C for *A. nigriscutis* (Table 14). The development rates (1/d) for *A. lacertosa* and *A. nigriscutis* were linear with temperature as shown in Figures 15 and 16 respectively. Based on the regression analysis, the lower developmental threshold estimate for *A. lacertosa* and *A. nigriscutis* are 8.3 °C and 10.1 °C respectively (Table 15). The degree-days required for adult emergence are 448 for *A. lacertosa* and 425 for *A. nigriscutis* based on the reciprocal of the slope of the linear regression.

Phenology Model
Pooled data and derived non-linear models describing proportional seasonal abundance as a function of accumulated degree-days for *A. lacertosa* and *A. nigriscutis*, are shown in Figures 17 and 18. Pooled data (Table 17) resulted in four "location years" as described by Legg et al. (2002). Model equations and R^2 value for non-linear models are provided in Table 16. The estimated accumulated degree-days to peak abundance of *A. lacertosa* is 513 (based on a lower developmental threshold of 8.3 °C). The estimated accumulated degree-days to peak abundance of *A. nigriscutis* is 610 (based on a lower developmental threshold of 10.1 °C). Calculations, estimates and standard errors for predicting the number of accumulated degree-days at peak abundance are listed in Table 17.

Displaying Models Spatially
Maps displaying estimated average dates to peak abundance for *A. lacertosa* and *A. nigriscutis* are displayed in Figure 21. Average date to peak abundance for *A. lacertosa* ranged from June 17^{th} to July 22^{nd} depending on location in the state. Average date to peak abundance for *A. nigriscutis* ranged from June 27^{th} to August 8^{th}, approximately 10 days later statewide than *A. lacertosa* in similar geographic locations. Peak emergence occurred earlier in the year in the southern part of the state for both species and became progressively later as you move north and east in the state.

Discussion

Developmental Rates and Lower Developmental Threshold Determination.
We chose to study the development time of overwintering third instar larvae to adult emergence as a measure of rate of development for *A. lacertosa* and *A.nigriscutis*. It is this development stage which determines timing of adult emergence in the field. *Aphthona lacertosa* and *A. nigriscutis* both emerged with great predictability when held at constant temperatures, shown by the high R^2 values for the linear regressions for each species (Table 15). The linear models for *A. lacertosa* and *A. nigriscutis* were similar in slope but had distinctly different intercepts (Figures 15 and 16). This difference is clear in the lower developmental threshold estimates for each species (Table 15). *Aphthona lacertosa* emerged earlier from each constant temperature than *A. nigriscutis*. In the field, *A. nigriscutis* tends to emerge later in the season than *A. lacertosa*. This observation is supported by estimates of required degree-days and lower

developmental thresholds. Although *A. nigriscutis* has a lower number of degree-days required for adult emergence than *A. lacertosa*, it takes longer for *A. nigriscutis* to complete development because its lower developmental threshold value is 1.8 °C higher than *A. lacertosa*. The lower developmental threshold and required degree-day estimates can be used to develop predictions of *Aphthona* spp. emergence in the field.

Phenology Model

Models predicting seasonal abundance of *A. lacertosa* and *A. nigriscutis* were developed using field-collected insects. With estimated lower developmental thresholds and ADD for each species, the model results can be used to time sampling and insect collection for redistribution. The required degree-days to peak abundance from this field model differed from the values from lab study listed in Table 17. This may be due in part, to increased variation in the field-collected data. The field models are based on air temperatures collected from nearby weather stations. The insects, however, are in the soil and are affected by micro-climates that differ between sites. This can be seen in Figure 19, which depicts ADD by soil temperature at various sites. Although the lab-derived models provide useful information, we will use the field models for predicting *Aphthona* abundance in the field.

Displaying Models Spatially

Maps were developed to spatially display average dates to peak abundance for *A. lacertosa* and *A. nigriscutis*. The maps provide researchers and land managers information on when to collect agents for redistribution, assess population establishment and carry out other management strategies as part of an integrated pest management program. The maps are based on normal temperatures, which will not account for the year to year variation in degree-day accumulations or the varying microclimates at each site. A site with a south facing slope and sandy soil, is an example of a site that peak abundance may occur earlier than predicted by the spatial model. It is important to only use the maps as a guide. As a rule, it is better to check the sites at the earliest date of the predictions to ensure peak abundance is not missed.

Table 14. Mean days to emergence with standard errors (d) and developmental rates (1/d) for *Aphthona lacertosa* and *A. nigriscutis* held at constant temperatures (T^{0}C).

	A. lacertosa			***A. nigriscutris***		
T (°C)	No. Adults	d	1/d	No. Adults	d	1/d
15	856	68.6±0.32	0.015	34	84.3±1.12	0.012
18	1432	46.5±0.27	0.022	50	56.0±1.37	0.018
21	161	34.7±0.33	0.029	48	37.1±0.77	0.027
22.5	410	31.4±0.21	0.032	45	36.1±1.05	0.028
24	226	28.4±0.22	0.035	17	31.2±1.48	0.032
26	270	25.6±0.23	0.039	88	26.1±0.47	0.038

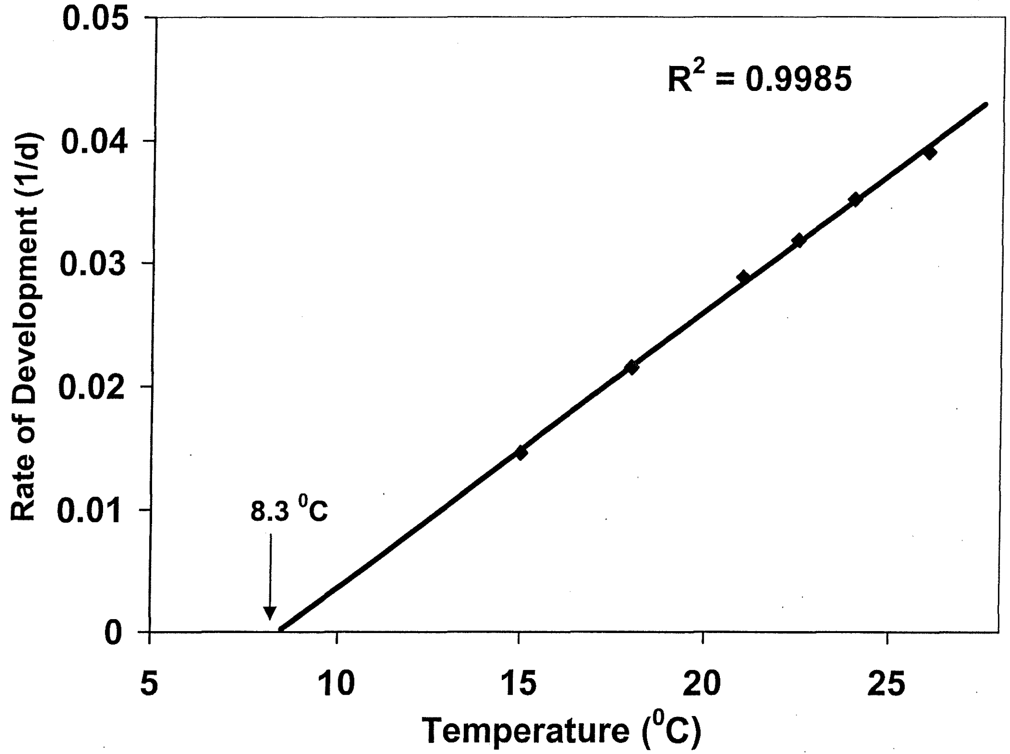

Figure 15. *Aphthona lacertosa* rate of development based on constant temperatures. Lower developmental threshold estimate indicated by arrow.

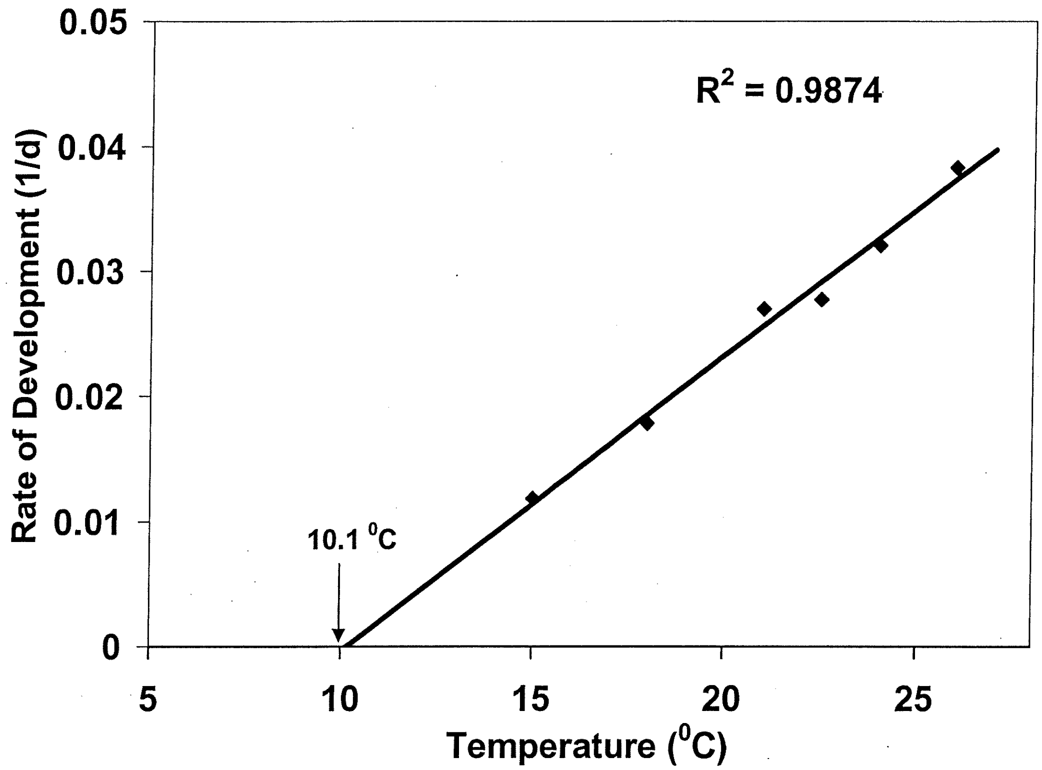

Figure 16. *Aphthona nigriscutis* rate of development based on constant temperatures. Lower developmental threshold estimate indicated by arrow.

Table 15. Estimates and constants from the regression of rate of development (1/d) on temperature (^{0}C), lower developmental threshold estimate (T_0), and required degree-days (DD) for development of *Aphthona lacertosa* and *A. nigriscutis*.

Aphthona lacertosa

	Y-Intercept	Slope	R^2	T_0 (°C)	DD (°C-day)
	-0.0186	0.0022	0.9985	8.33	448
SE	(0.0009)	(0.00002)		(0.255)	(8.58)

Aphthona nigriscutis

	Y-Intercept	Slope	R^2	T_0 (°C)	DD (°C-day)
	-0.0238	0.0024	0.9874	10.14	425
SE	(0.0031)	(0.0001)		(0.6921)	(25.44)

Table 16. Equations of fitted model and prediction for number of accumulated degree-days at peak abundance.

Aphthona lacertosa

Fitted model

$y = 5E\text{-}09x^3 - 1E\text{-}05x^2 + 0.0073x - 1.3099$
$R^2 = .48$

Equation predicting ADD at peak abundance
Peak = $-2(.00001)- (.00001^2 - 12(.0073)(5E\text{-}09))^{1/2}/6(5E\text{-}09)$
Peak = 513 ADD
SE = 19.9 ADD

Aphthona nigriscutis

Fitted model

$y = -7E\text{-}09x^3 + 9E\text{-}06x^2 - 0.0027x + 0.2249$
$R^2 = .40$

Equation predicting ADD at peak abundance

Peak = $-2(9E\text{-}06)- (9E\text{-}06^2 - 12(0.0077)(-7E\text{-}09))^{1/2}/6(-7E\text{-}09)$
Peak = 610 ADD
SE = 26.7 ADD

Table. 17. Percent maximum capture and associated accumulated degree-days (°C) for *A. lacertosa* and *A. nigriscutis*. Data are averages by sampling date, pooled by year and county (n=18 sampling sites in Clay County and n=8 sampling sites in Otter Tail County).

		A. Lacertosa		*A. nigriscutis*	
County	Sample Date	ADD	% Max Capture	ADD	% Max Capture
Clay	6/7/2000	443.07	0.09	324.84	0.00
Clay	6/15/2000	545.73	0.14	407.80	0.01
Clay	6/22/2000	607.65	0.27	455.58	0.04
Clay	6/28/2000	671.79	0.15	504.75	0.10
Clay	7/5/2000	770.33	0.15	586.09	0.32
Clay	7/11/2000	858.64	0.08	659.41	0.19
Clay	7/19/2000	956.85	0.06	738.50	0.18
Clay	7/26/2000	1046.11	0.07	811.84	0.16
Otter Tail	6/8/2000	459.86	0.02	335.33	0.03
Otter Tail	6/14/2000	536.23	0.12	396.69	0.02
Otter Tail	6/22/2000	603.77	0.24	446.64	0.02
Otter Tail	6/29/2000	679.53	0.30	504.91	0.32
Otter Tail	7/6/2000	784.46	0.17	592.33	0.23
Otter Tail	7/11/2000	861.16	0.06	656.53	0.18
Otter Tail	7/20/2000	968.21	0.05	741.38	0.15
Otter Tail	7/27/2000	1055.91	0.03	811.59	0.08
Clay	6/6/2001	376.90	0.01	284.44	0.00
Clay	6/14/2001	471.50	0.04	359.04	0.01
Clay	6/20/2001	528.15	0.13	401.26	0.02
Clay	6/27/2001	633.35	0.23	490.13	0.08
Clay	7/2/2001	707.71	0.11	553.24	0.03
Clay	7/11/2001	679.53	0.26	662.81	0.36
Clay	7/18/2001	957.47	0.16	763.57	0.34
Clay	7/25/2001	1068.23	0.06	856.83	0.15
Otter Tail	6/7/2001	372.39	0.00	277.47	0.00
Otter Tail	6/15/2001	470.05	0.07	355.12	0.00
Otter Tail	6/21/2001	521.97	0.27	392.95	0.00
Otter Tail	6/28/2001	635.23	0.33	488.71	0.31
Otter Tail	7/3/2001	699.98	0.08	541.82	0.00
Otter Tail	7/12/2001	831.20	0.13	650.54	0.63
Otter Tail	7/18/2001	933.12	0.09	737.46	0.22
Otter Tail	7/25/2001	1044.16	0.04	831.00	0.00

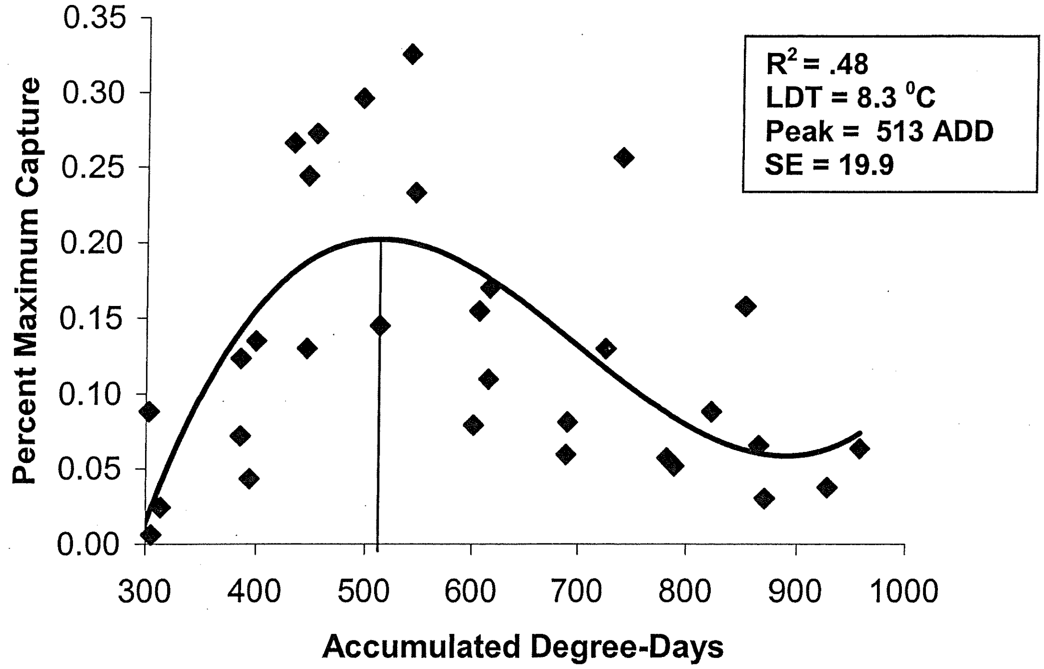

Figure 17. Regression and fitted model of percent maximum capture and accumulated degree-days for *Aphthona lacertosa*. Estimation of peak abundance marked by vertical line.

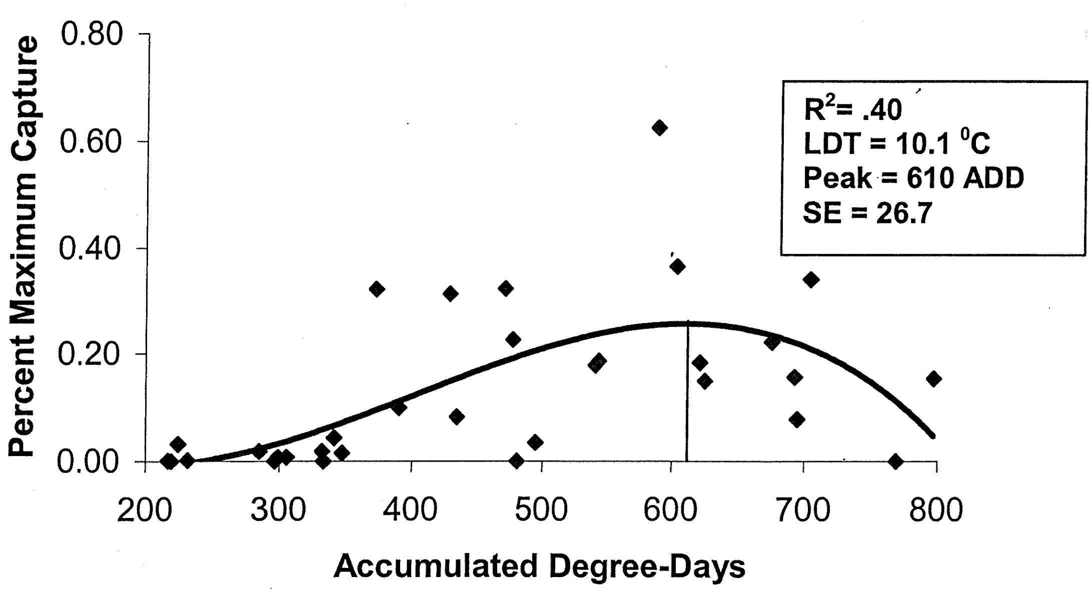

Figure 18. Regression and fitted model of percent maximum capture and accumulated degree-days for *Aphthona nigriscutis*. Estimation of peak abundance marked by vertical line.

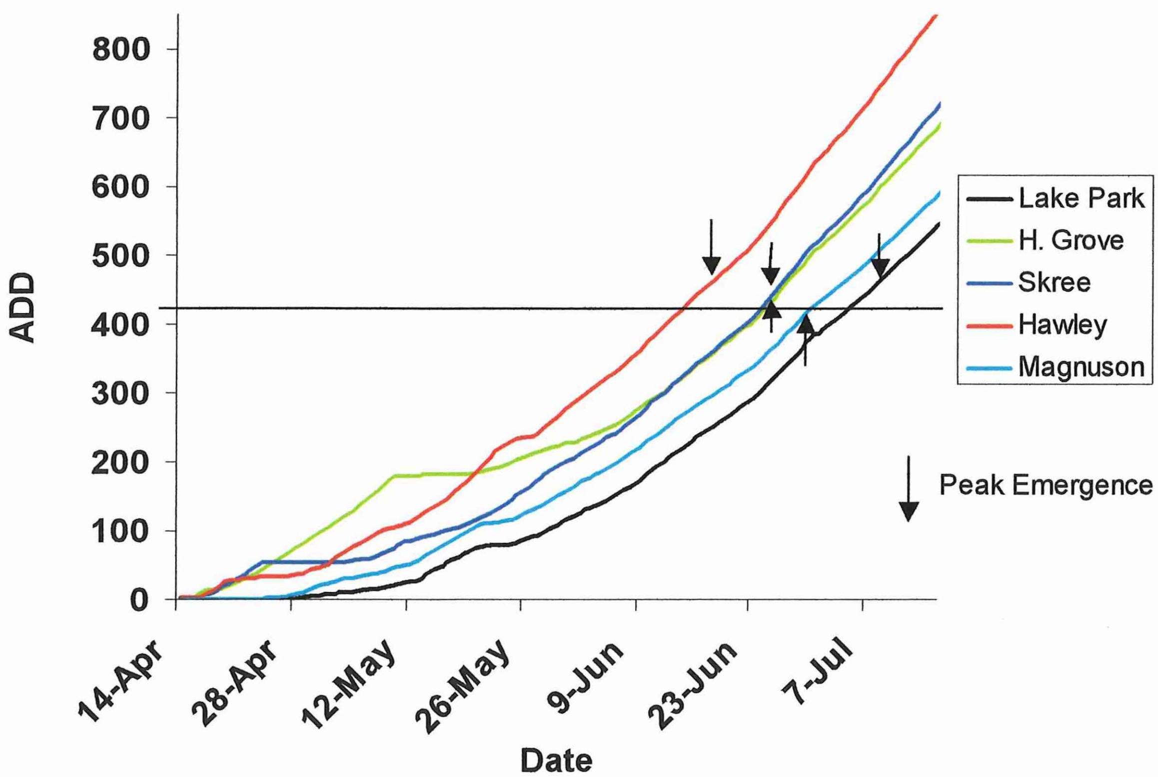

Figure 19. Accumulated degree-days based on soil temperature and peak abundance of *A. lacertosa* in 2001. ADD based on lower developmental threshhold of 8.3 °C.

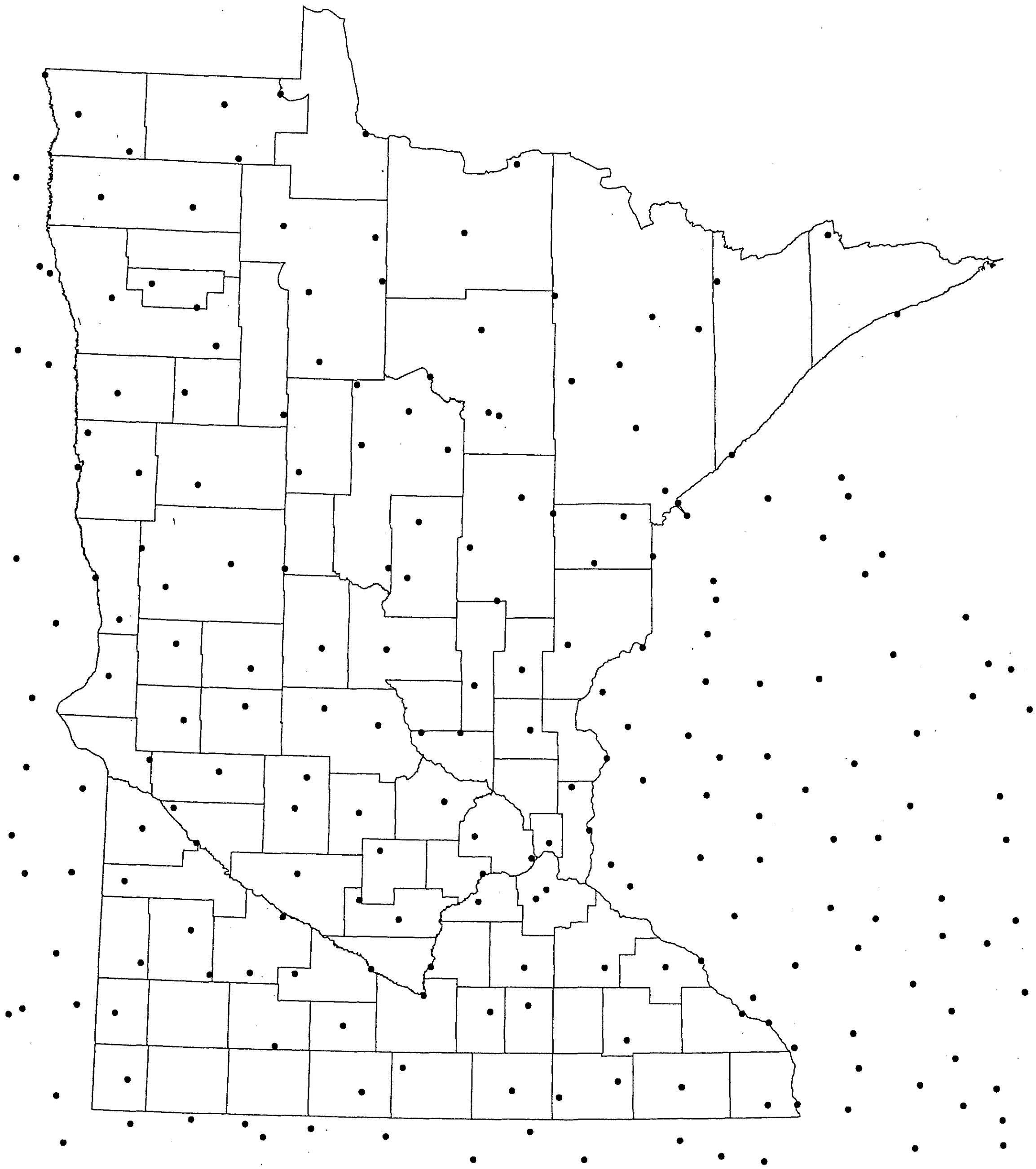

Figure 20. Locations of weather stations used in map development based on peak abundance. Thirty year average temperature data (normal) were obtained for each weather station.

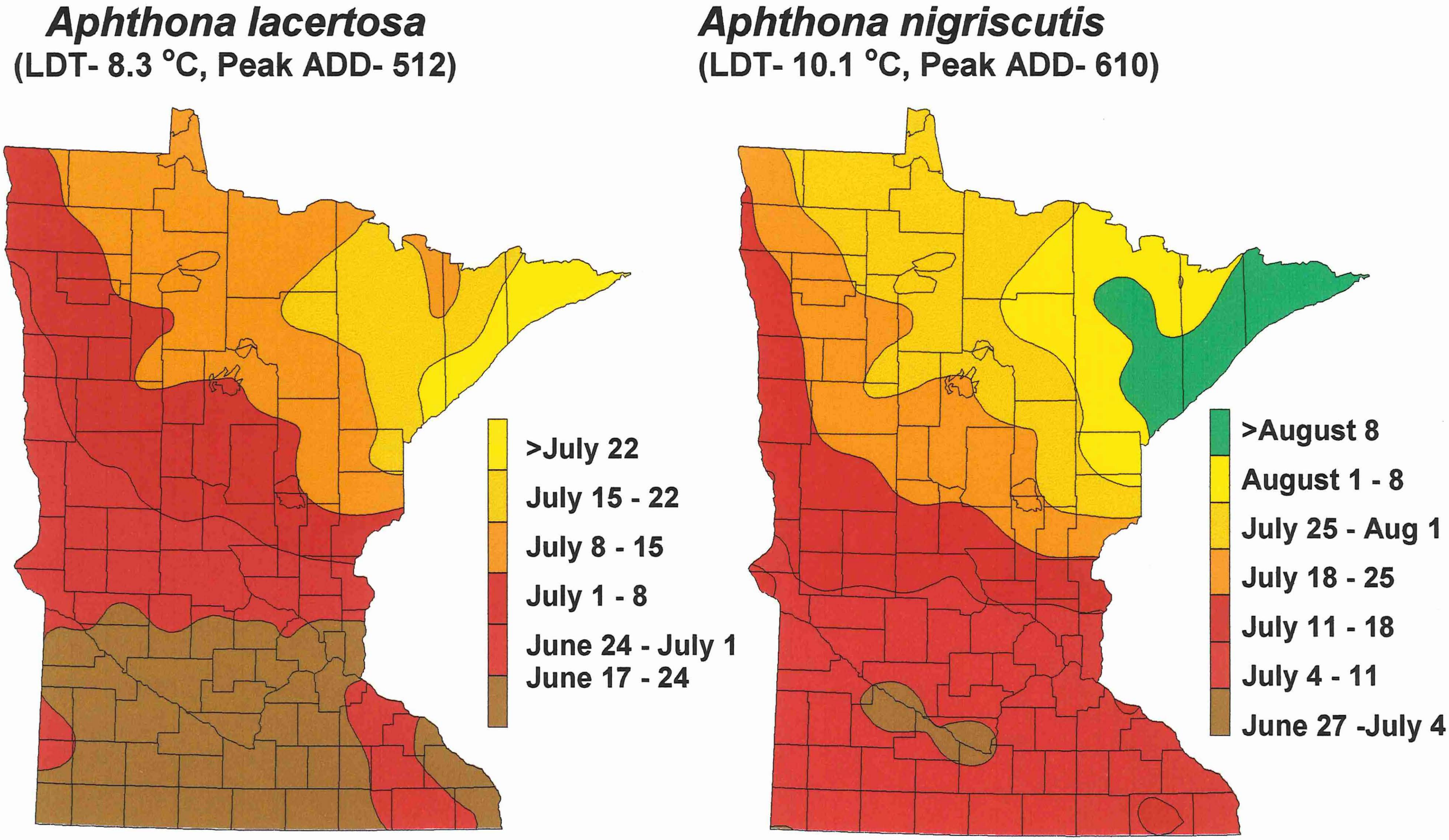

Figure 21. Mean date to peak abundance in Minnesota for *Aphthona lacertosa* and *A. nigriscutis*.

Summary

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Leafy spurge is a Eurasian perennial plant that seriously impacts native plants, wildlife and grazing land for cattle and horses. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge. Some of the species, however, have had difficulty establishing and have not contributed to control success. Factors that may affect insect establishment include soil type, soil moisture, leafy spurge density, leafy spurge biotype, vegetation type, litter cover, release quantity, and interspecific competition.

The results suggest that *A. lacertosa* is the most effective species in controlling leafy spurge in Minnesota. *Aphthona lacertosa* established at 100% of the release sites and significantly reduced leafy spurge by 63% across all sites studied. *Aphthona nigriscutis* established at 73% of the study sites, but at significantly lower densities than *A. lacertosa*. *Aphthona nigriscutis* most likely contributed to the control success at sites where both species occurred. Other introduced *Aphthona* species are difficult to locate in Minnesota and contributed little to the overall control success occurring statewide. Correlations between biotic/abiotic factors and flea beetle density were not clearly evident. Only soil texture seemed to affect *A. lacertosa* densities, which may not have biological significance. *Aphthona nigriscutis* seemed to establish best on drier sites, although our results were inconclusive on this topic. *Aphthona nigriscutis* was observed at highest densities in dry sites with either sand or sandy loam soils.

Early indications showed that interspecific competition between *A. lacertosa* and *A. nigriscutis* was not affecting flea beetle populations. We speculate that habitat type and leafy spurge densities are the predominant factors that affect beetle establishment. Small release quantities (<500 beetles) may have contributed to lack of establishment on early releases made in Minnesota. Although the beetles became established in all treatments, all treatment populations were small one year after release. This suggests that the current practice of releasing >4,000 flea beetles per site will increase establishment, reproduction and eventual redistribution.

Phenology models predicting peak emergence of *A. lacertosa* and *A. nigriscutis* were developed to provide information to resource managers on when to collect beetles for redistribution. The model results can be used in two ways. First, the lower developmental threshold (LDT) and accumulated degree-days (ADD) to peak emergence can be used to calculate current ADD with local weather station temperature data. The University of Minnesota Climatology Working Group provides an interactive website that will calculate ADD based on a predetermined LDT for ~30 locations statewide. This allows resource managers to track degree-day accumulation and plan collection events on or near the predicted ADD for each species. The second method is to use maps developed on 30-year temperature data that estimated peak abundance dates in the field.

Recommendations

We recommend that *A. lacertosa* be used as the primary agent for control of leafy spurge in Minnesota. We also recommend that *A. nigriscutis* continue to be redistributed statewide, particularly to sites where *A. lacertosa* may not do well. Mixed colonies are preferred for sites that are very dry with sandy soils. Efforts to collect and redistribute *A. cyparissae* and *A. flava* should be considered low priority, unless field populations become highly abundant.

The current practice of releasing >4,000 beetles per leafy spurge infestation, should be continued. If flea beetle abundance becomes low, smaller release quantities can be released with some success.

We recommend that release sites be visited starting two years post release. Our results show that flea beetle populations increase dramatically in the second or third year. Leafy spurge reduction, on average, also begins to decline rapidly three years post release, but may occur as early as two years post release. Importance of monitoring two years post release are two-fold, first to monitor success of the biocontrol release and secondly to determine if flea beetle populations are large enough that a collection can be made for redistribution.

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Appendix A: *Aphthona* spp. images

Aphthona abdominalis

Aphthona cyparissiae

Aphthona czwalinae

Aphthona flava

Aphthona lacertosa

Aphthona nigriscutis

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Appendix B: List of Plant Names

List of Plant Species for the Assessment of Biotic and Abiotic Factors Affecting the Establishment of *Aphthona* spp. – Intensive Study

Acer spp.
Achillea millefolium
Agropyron repens
Anemone canadensis
Anemone quinquefolia
Apocynum androsaemifolium
Apocynum cannabinum
Artemisia ludoviciana
Asclepias syriaca
Aster spp.
Bromus inermis
Calylophus serrulatus
Carduus acanthoides
Carduus nutans
Carex spp.
Centaurea maculosa
Cirsium arvense
Convolvulus arvensis
Conyza canadensis
Coronillia varia
Dactylis glomerata
Digitaria ischaemum
Equisetum arvense
Eupatorium rugosum
Fragaria virginiana
Galium aparine
Lappula spp.
Lilium philadelphicum
Lychnis alba
Lysimachia spp.
Medicago sativa
Melilotus alba
Monarda fistulosa
Oxalis stricta
Panicum capillare
Panicum spp.
Panicum virgatum
Pastinaca sativa
Phalaris arundinacea
Pheum pratense
Poa pratensis
Potentilla anserina
Quercus spp.
Rhus glabra
Rosa spp.
Rudbeckia hirta
Senecio integerrimus
Setaria spp.
Solidago spp.
Sonchus arvensis
Sporobolus heterolepis
Stipa spatea
Symphoricarops orbiculatus
Symphoricarpos albus
Thalictrum spp.
Trifolium pratense
Trifolium repens
Verbascum thapsus
Vicia angustifolia
Viola canadensis
Viola pratincola
Zizia aurea

List of Plant Species for the Assessment of Biotic and Abiotic Factors Affecting the Establishment of *Aphthona* spp. – Extensive Study

Abutilon theophrasti
Acer ginnala
Acer negundo
Acer spp.
Achillea millefolium
Agropyron repens
Ambrosia psilostachya
Ambrosia trifida
Amorpha canescens
Anaphalis margaritacea
Andropogon geradii
Andropogon scoparius
Asclepias syriaca
Aster ericoides
Aster spp.
Aster umbellatus
Berteroa incana
Bromus inermis
Carduus acanthoides
Carex spp.
Chenopodium album
Chrysanthemum leucanthemum
Cirsium altissimum
Cirsium arvense
Cirsium flodmanii
Comandra umbellata
Convolvulus arvensis
Cornus spp.
Coronillia varia
Daisy Fleabane
Daucus carota
Equisetum arvense
Fragaria virginiana
Galium aparine
Helianthus spp.
Kochia scoperia
Koelaria pyramidata
Lotus corniculatus
Lupinus perennis
Lychnis alba
Medicago sativa
Melilotus alba
Melilotus officinalis
Oenothera biennis
Panicum capillare
Panicum oligosanthes
Parthenocissus vitacea
Phalaris arundinacea
Pheum pratense
Physalis spp.
Pinus spp.
Poa pratensis
Polygonatum biflorum
Populus spp.
Potentilla recta
Quercus spp.
Rhus glabra
Rhus radicans
Rosa arkansana
Rosa spp.
Rubus spp.
Rudbeckia hirta
Salix spp.
Setaria viridis
Solidago spp.
Sonchus arvensis
Stachys palustris
Symphoricarops orbiculatus
Tanacetum vulgare
Taraxacum officinale
Teucrium canadense
Tradescantia occidentalis
Trifolium pratense
Trifolium repens
Ulmus pumila
Verbascum thapsus
Verbena hastata
Verbena stricta
Vicia angustifolia
Viola canadensis
Viola pratincola
Vitis riparia

List of Plant Species Found in the Effect of Release Quantity on Establishment and Control by *Aphthona lacertosa* and *A. nigriscutis*

Asclepias syriaca
Aster spp.
Bidens spp.
Bromus inermis
Carduus nutans
Chenopodium album
Cirsium arvense
Convolvulus arvensis
Conyza canadensis
Fragaria virginiana
Galium aparine
Lychnis alba
Melilotus officinalis
Nepeta cataria
Oxalis stricta
Pastinaca sativa
Phalaris arundinacea
Physalis spp.
Plantago major
Poa pratensis
Polygonum spp.
Ribes spp.
Rosa spp.
Rubus spp.
Saponaria officinalis
Solidago spp.
Sonchus arvensis
Trifolium pratense
Verbascum thapsus
Vicia angustifolia

Appendix C: Seedbank Study

Introduction

Biological control of leafy spurge using flea beetles has proven highly effective. As spurge density decreases, *Aphthona* spp. population decreases correspondingly. However, a leafy spurge seedbank remains from the prior infestation generating the potential for reestablishment by seed. Since biological control of leafy spurge began in the last 15 years, information on the long term effects of leafy spurge control is not available.

Leafy spurge reproduces by both seed and vegetative root buds. Leafy spurge plants produce high numbers of seed (an average of 140 seeds per stem) and seeds can remain viable in the soil for at least eight years (Lym et al. 1998). Mature seed can be ejected a distance of 4.5 m from the parental plant (Carmichael and Selbo 1998). Seed dispersal can be aided by wildlife, wind, water, and humans. Germination of leafy spurge seeds may result in infestation of a site considered controlled with germination occurring predominantly in late May or early June. Seedlings can reproduce vegetatively within 7 to 10 days after emergence and a patch of leafy spurge can spread vegetatively from 1 to 3 feet per year (Lym et al. 1998).

The purpose of this study is to evaluate three sites formerly infested with leafy spurge for the potential of reestablishment by seed. The spurge infestations were controlled using *Aphthona* spp. as biological control agents.

Materials and Methods

Three sites were selected where leafy spurge density was reduced to few remaining stems: Flandrau F (Brown County), Skree A (Clay County), and Forget Me Not Island (Becker County). Leafy spurge vegetation data were taken during the summer of 2001. Ten 0.18 m^2 samples per site were selected randomly using a square. For each sample the number of flowering spurge plants, number of non-flowering spurge plants, and height of the 5 tallest spurge plants were recorded. Soil was collected in the early spring (May 2001). The soil surface debris was cleared then soil collected to a depth of 10 cm using a soil probe or trowel and placed in plastic bags and frozen until processing. Ten random samples were collected per site.

Soil was dried for three days on paper towels. Seeds were extracted using an adapted method from Ball and Miller (1989). In a plastic container, 200 ml of magnesium sulfate solution (containing 10 g of sodium hexametaphosphate, 5 g of sodium bicarbonate, and 25 g of magnesium sulfate dissolved in 200 ml of tap water) per 100 g soil were stirred continuously for 2 minutes. The slurry was allowed to settle for 5 minutes. The seed and organic matter fraction floated on the surface and was decanted onto a 50 mesh sieve. The floatation/separation procedure was repeated four times using the same magnesium sulfate solution. Soil was washed from the seed and organic matter fraction on the sieve with tap water then transferred to filter paper in a Petri dish to dry. Intact, whole leafy spurge seeds were hand separated from remaining material and counted.

Results

There were no leafy spurge plants at Flandrau F and densities were very low at both Forget Me Not Island and Skree A. Mean leafy spurge stem densities ranged from 0.00 to 16.8 and 0.0 to 18.0 stems/m^2 for flowering and non-flowering respectively (Table 18). Mean heights ranged from 17 to 55.6 cm with Skree A having the highest mean spurge height.

Leafy spurge seed was found at all sites. One hundred percent of Forget Me Not Island, 60% of Skree A, and 50% of Flandrau F samples contained spurge seed. Forget Me Not Island had the highest mean spurge seed density followed by Flandrau F and Skree A respectively (Fig. 22).

Discussion

There was no correlation between the leafy spurge stem density and seed density. The spurge seed density varied by site (Fig. 22). This variation may be due to the time length and severity of the previous spurge infestation or factors affecting the longevity of seeds in the soil such as predation by insects and rodents.

Most of the samples from all sites contained spurge seed indicating that spurge seed is fairly well distributed throughout the sites. Extrapolated, the spurge seed density would be 4,214, 1,643, and 1,000 seeds per m^2 for Forget Me Not Island, Flandrau A, and Skree A respectively. The potential for reinfestation exists at all three sites. Thus, it is essential to monitor leafy spurge and *Aphthona* spp. populations after initial control is achieved.

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Table 18. Number of leafy spurge stems per m^2 during summer 2001.

Site	Flowering	Non-flowering	Total	Mean Height
Skree A	16.8±5.16	18.0±5.85	34.8±10.43	45.2±3.08
FMN Island	0.0±0.00	3.3±1.89	3.3±1.89	32.89±8.04
Flandrau F	0.0±0.00	0.0±0.00	0.0±0.00	NA

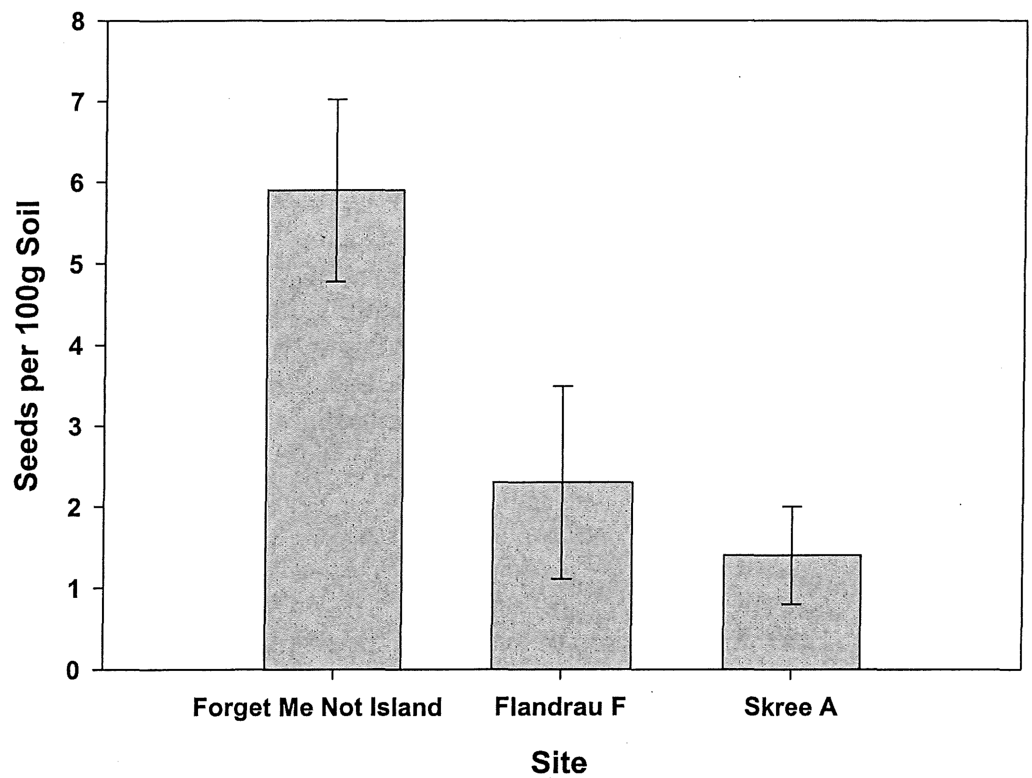

Figure 22. Mean leafy spurge seed density with standard error at three separate locations.